ANALYSIS OF IMMUNOHISTOCHEMICAL EXPRESSION OF $p^{16INK4a}$ IN PRENEOPLASTIC AND NEOPLASTIC SQUAMOUS CELL LESIONS OF CERVIX

Dissertation submitted in
Partial fulfillment of the regulations required for the award of

M.D. DEGREE

in

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

DECLARATION

I hereby declare that the dissertation entitled **"Analysis of Immunohistochemical Expression of $p^{16INK4a}$ in preneoplastic and neoplastic squamous cell lesions of cervix**" was done by me in the Department of Pathology, Chengalpattu Medical College from June 2014-August 2015 under the guidance and supervision of **Dr.S.Ravi, M.D**., Professor and Head, Department of Pathology, Chengalpattu Medical College.

This dissertation is submitted to the Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D. Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place:

Date: **Dr.P.Sakunthala**

CERTIFICATE FROM THE GUIDE

This is to certify that the dissertation entitled "**Analysis of Immunohistochemical Expression of $p^{16INK4a}$ in preneoplastic and neoplastic squamous cell lesions of cervix**" submitted by the candidate Dr.P.Sakunthala in partial fulfillment for the award of the degree of Doctor of Medicine in Pathology by The Tamilnadu Dr.M.G.R .Medical University, Chennai is a record of original work done by her under my guidance and supervision in the Department of Pathology, Chengalpattu Medical College, Chengalpattu during the academic year 2013-16.

Place:Chengalpattu
Date:

Dr.S.Ravi, M.D.,
Professor and Head,
Department of Pathology,
Chengalpattu Medical College,
Chengalpattu.

CERTIFICATE

This is to certify that the dissertation entitled "**Analysis of Immunohistochemical Expression of $p^{16INK4a}$ in preneoplastic and neoplastic squamous cell lesions of cervix"** is a record of bonafide work done by **Dr.P.Sakunthala** in the Department of Pathology, Chengalpattu Medical College, Chengalpattu under the supervision of **Dr. S. Ravi, M.D.,** Professor and Head , Department of Pathology and submitted in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai. This work has not previously formed the basis for the award of a degree or diploma.

Dr.K.Muthuraj, M.S .,
Dean,
Chengalpattu medical college,
Chengalpattu.

Dr.S.Ravi, M.D.,
Professor and Head,
Department of Pathology,
Chengalpattu Medical College,
Chengalpattu.

INSTITUTIONAL ETHICS COMMITTEE

CHENGALPATTU MEDICAL COLLEGE , CHENGALPATTU

APPROVAL OF ETHICAL COMMITTEE

To

Dr.P.Sakunthala,
MD Pathology (2nd Year),
Chengalpattu Medical College,
Chengalpattu

Dear Dr.

The Institutional Ethical Committee of Chengalpattu Medical College reviewed and discussed your application to conduct the clinical / dissertation work entitled

ANALYSIS OF IMMUNOHISTOCHEMICAL EXPRESSION OF P16INK4A IN PRENEOPLASTIC AND NEOPLASTIC SQUAMOUS CELL LESIONS OF CERVIX.

ON 11.06.2014

The following documents reviewed

1. Trial protocol, dated _____version no
2. Patient information sheet and informed consent form in English and / or
   vernacular language.
3. Investigators Brochure, dated _____version
4. Principal Investigators current CV
5. Investigators undertaking

The following members of the Ethics committee were present at the meeting held on

Date 11.06.2014 Time 11.30 Noon Place Chengalpattu Medical College

Approved _____ Chairman Ethics Committee

_____ Member secretary of Ethics Committee.

Name of each member with designation:-

Clinical Members

1. Dr.R.Muthuselvan MD.,
   Prof & HOD of Medicine, CHMC

2. Dr.C.Srinivasan MD.,
   Prof & HOD of Surgery, CHMC

Biological Scientist

3. Dr.K.Baskaran MD.,
   Asso Prof of Pharmacology, CHMC

Non Clinical Member

4. Dr.P.Parasakthi MD
   Prof & HOD of Forensic Medicine,CHMC

5. Member from Nongovernmental
   Voluntary Organisation : Mr.P.Durairaj

6. Philosopher : Mr.K.S.Ramprasad

7. Lawyer : Lr. I. M. Karimala Basha

8. Layperson : Mr.Dilli

We approve the clinical trial to be conducted in its presented form

The Institutional Ethics Committee expects to be informed about the progress of the study and any SAE occurring in the course of the study, any changes in protocol and patient information / informed consent and asks to provide copy of final report.

Yours sincerely

11/6/14

Member secretary, Ethics Committee

ACKNOWLEDGEMENT

To think with, I thank the almighty GOD in making this project a successful one.

I express my deep gratitude to **Dr.K. Muthuraj, M.S.**, Dean, Chengalpattu Medical College, for granting me permission to undertake this study.

I profusely thank and express my sincere gratitude to **Dr**.**S.Ravi, M.D**., Professor and Head, Department of Pathology, Chengalpattu Medical College, for having suggested this topic for dissertation and for having rendered his valuable support and encouragement without which this project work would not have been feasible.

I wish to record my sincere thanks to **Dr.I.Vijay sathish kumar**,**M.D., Dr.S.Sasikala, M.D**., **Dr.K.R.Mohan, M.D., Dr.S.Premalatha, M.D.,** Associate Professors, Department of Pathology, Chengalpattu Medical College, for their constant support and encouragement throughout my work.

I express my heartfelt thanks to **Dr.G.Selvambigai, M.D.**, Associate Professor, Department of Pathology, Government omanthoorar Medical College, chennai for her support and valuable advice during the course of this study.

I also wish to record my sincere thanks to **Dr. M.Kuzhalmozhi, M.D., Dr. S.Surya, M.D., Dr. V.palaniappan, M.D., Dr. M.Malathy, MD.,**

Dr. G.Devi Priya., Dr. S.Rohini Priya., Dr. V.Dhamotharan, MD., Assistant Professors **Dr. T.Renuga Sarojini, DCP.,** Tutor of the pathology,Department of Pathology, Chengalpattu Medical College, for their constant support and encouragement throughout my work.

I extend my heartfelt thanks to all my colleagues for their timely help, comments and support.

I thank all the technical staff in the Department of Pathology, Chengalpattu Medical College, for their sincere and timely technical assistance.

Also, I am indebted to my husband **Mr.R.Pradeep kumar** for his constant support, encouraging words and source of strength all the way through this endeavour.

To my lovable family members, I express my gratitude for their extreme patience and tireless support while pursuing this study.

Last, but not the least I am indebted to all the patients who made it possible for me to carry out this study. I extend all my support and wishes for their healthy lives.

Dr.P.Sakunthala

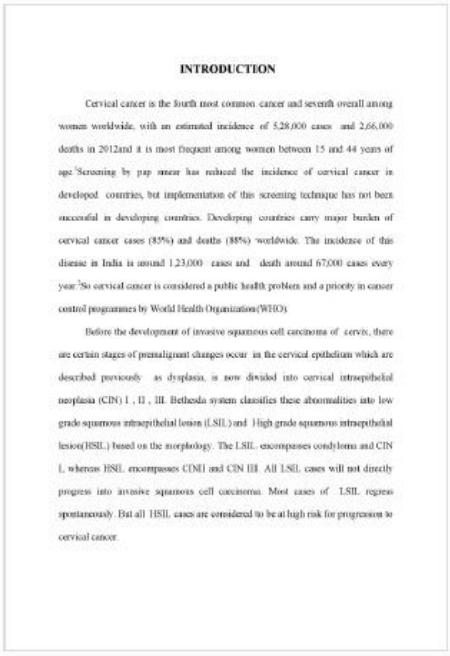
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

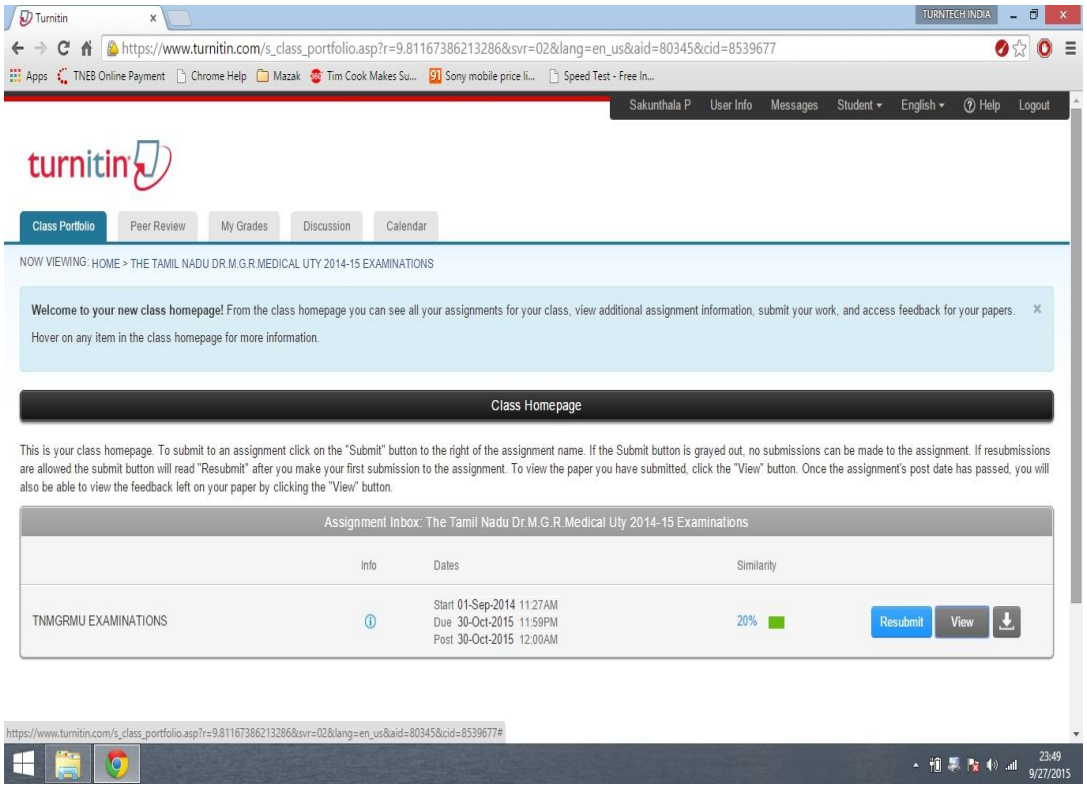
Submission author:	Sakunthala P
Assignment title:	TNMGRMU EXAMINATIONS
Submission title:	Analysis of Immunohistochemical E..
File name:	sakunthala_dissertation.docx
File size:	2.38M
Page count:	110
Word count:	15,364
Character count:	85,712
Submission date:	27-Sep-2015 11:37PM
Submission ID:	569598597



INTRODUCTION

Cervical cancer is the fourth most common cancer and seventh overall among women worldwide, with an estimated incidence of 5,28,000 cases and 2,66,000 deaths in 2012and it is most frequent among women between 15 and 44 years of age.[1]Screening by pap smear has reduced the incidence of cervical cancer in developed countries, but implementation of this screening technique has not been successful in developing countries. Developing countries carry major burden of cervical cancer cases (85%) and deaths (88%) worldwide. The incidence of this disease in India is around 1,23,000 cases and death around 67,000 cases every year.[2]So cervical cancer is considered a public health problem and a priority in cancer control programmes by World Health Organization(WHO).

Before the development of invasive squamous cell carcinoma of cervix, there are certain stages of preinvasive changes occur in the cervical epithelium which are described previously as dysplasia, is now divided into cervical intraepithelial neoplasia (CIN) I , II , III. Bethesda system classifies these abnormalities into low grade squamous intraepithelial lesion (LSIL) and High grade squamous intraepithelial lesion(HSIL) based on the morphology. The LSIL encompasses condyloma and CIN I, whereas HSIL encompasses CDNI and CIN III. All LSIL cases will not directly progress into invasive squamous cell carcinoma. Most cases of LSIL regress spontaneously. But all HSIL cases are considered to be at high risk for progression to cervical cancer.


Turnitin
TURNTECH INDIA
https://www.turnitin.com/s_class_portfolio.asp?r=9.81167386213286&svr=02&lang=en_us&aid=80345&cid=8539677
Apps
TNEB Online Payment
Chrome Help
Mazak
Tim Cook Makes Su...
Sony mobile price li...
Speed Test - Free In...
Sakunthala P
User Info
Messages
Student
English
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2014-15 EXAMINATIONS
Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations
Info
Dates
Similarity
TNMGRMU EXAMINATIONS
Start 01-Sep-2014 11:27AM
Due 30-Oct-2015 11:59PM
Post 30-Oct-2015 12:00AM
20%
Resubmit
View
https://www.turnitin.com/s_class_portfolio.asp?r=9.81167386213286&svr=02&lang=en_us&aid=80345&cid=8539677#
23:49
9/27/2015

CONTENTS

LIST OF TABLES

18	Comparison of incidence of parity among preneoplastic and neoplastic lesions of cervix
19	Comparison of incidence of various symptoms in CIN and SCC
20	Comparison of incidence of CIN with other studies
21	Comparison of distribution of various histological subtypes of SCC
22	Comparison of P16 positivity in CIN and SCC with other studies
23	Comparing Grade of P16 expression in squamous cell lesions of cervix
24	Correlation between P16 reaction intensity and histological diagnosis with other studies

LIST OF FIGURES

ABSTRACT

BACKGROUND

Cervical cancer is the fourth most common cancer affecting women worldwide. Pap smear screening and histopathological interpretation of cervical biopsy has significantly reduced the number of deaths due to cervical cancer. However, they provide little or no information regarding the association of HPV in precancerous lesion and invasive cervical cancer. Hence the need to use biomarker to know the association of HPV in those lesions in order to predict the risk of progression or regression and prognosis. P^{16INKa} is a surrogate marker of HPV which fulfils all the above criteria.

OBJECTIVES

1. To evaluate the results of expression of p16INK4A in preneoplastic and neoplastic lesions of cervix in order to assess the association of HPV infection in those lesions.

2. To study the pattern of expression of p16 in various histological types of cervical squamous cell lesions by p16 immunohistochemistry.

3. To compare p16 expression in various histological types of cervical squamous cell lesions.

MATERIALS AND METHODS

Immunohistochemical analysis of p16expression was performed on 60 paraffin embedded tissue samples, obtained from cervical biopsy including 25CIN I, 5CIN II, 4CIN III and 26 SCC. Two parameters were evaluated in p16 expression: Percentage ofp16 positive cells and reaction intensity of p16 immunostaining. The p16 expression was graded as Negative, Grade 1, 2, 3 and its reaction intensity was graded as Negative , Weak, Moderate, Strong.

RESULTS

In the present study out of 60 cases, the incidence of squamous cell carcinoma constituted majority (44%).Among CIN group , CIN I constituted majority of the preneoplstic lesions of cervix.p16 expression was seen in 28% of CIN I, 80% of CIN II, all CIN III and all SCC cases. Only one CIN I case show showed grade3 staining and strong reaction intensity, but most of the CIN II(60%), CIN III(100%) and SCC(96.15%) cases showed grade 3 staining. In our study there was a statistically significant correlation between p16 expression, reaction intensity and lesion severity.

CONCLUSION

In the present study out of 60 cases, 68.33% of cases showed p16 positivity. In preneoplastic lesions, totally 44.12% of cases showed p16 positivity. In invasive squamous cell carcinoma cases, 100% cases showed p16 positivity. So p16 may be useful as an adjunct in histological sections to know the association of HPV in preneoplastic lesions lesions to predict the risk of progression of the disease and to plan proper treatment and in neoplastic lesions to predict the prognosis, since HPV negative SCC showed poor prognosis in literatures.

In our study p16 expression was correlated well with increasing grade of CIN. So p16 has significant implication in diagnostic, prognostic and preventive aspects of cervical cancer.

KEY WORDS

P16INK4A, Cervical intraepithelial neoplasia, Immunohistochemistry, Human papilloma virus.

INTRODUCTION

Cervical cancer is the fourth most common cancer and seventh overall among women worldwide, with an estimated incidence of 5,28,000 cases and 2,66,000 deaths in 2012 and it is most frequent among women between 15 and 44 years of age.[1]Screening by pap smear has reduced the incidence of cervical cancer in developed countries, but implementation of this screening technique has not been successful in developing countries. Developing countries carry major burden of cervical cancer cases (85%) and deaths (88%) worldwide. The incidence of this disease in India is around 1,23,000 cases and death around 67,000 cases every year.[2] So cervical cancer is considered as a public health problem and World Health Organization(WHO) gives priority to cervical cancer control programmes.

Before the development of invasive squamous cell carcinoma of cervix, there are certain stages of premalignant changes that occur in the cervical epithelium which are described previously as dysplasia. They are now divided into cervical intraepithelial neoplasia (CIN) I, II and III. Bethesda system classifies these abnormalities into low grade squamous intraepithelial lesion (LSIL) and High grade squamous intraepithelial lesion (HSIL) based on the morphology. The LSIL encompasses condyloma and CIN I, whereas HSIL encompasses CINII and CIN III. All LSIL cases will not directly progress into invasive squamous cell carcinoma. Most cases of LSIL regress spontaneously. But all HSIL cases are considered to be at high risk for progression to cervical cancer.

It is well known that the main causative factor for both precancerous and invasive cervical cancer is persistent infection with one or more oncogenic types of Human papilloma virus(HPV).[3]In addition to the infection with HPV, there are several cofactors have been associated with the increased risk of persistent infection of high-risk HPVs and progression to preneoplastic and neoplastic lesions of cervix, including Viral infections like HIV, Herpes simplex virus–2 (HSV-2)[4], Smoking[5], Dietary deficiencies[6], Immunosupression[7], Hormonal contraceptives, family history and sexually associated factors like multiple sexual partners, Early sexual activity, Sexually transmitted diseases, Multiple pregnancies.

Experimental studies have identified nearly 200 types of Human papilloma viruses, of those more than 40 have been identified in the genital tract.[8] These are divided into those with low risk and high risk categories based on the association with invasive cervical carcinoma.HPV16, 18, 31, 33 and 45 are examples of high-risk types, while HPV6 and 11 belong to the low-risk types. In a large epidemiological study conducted in India showed that genotypes 16 and 18 either alone or together were detected in 76.3% of cervical cancer cases followed by genotype 33.[9]

The two viral oncoproteins in HPV namely E6 and E7 are mainly responsible for the progression of neoplasm. The E6 oncoprotein of high risk HPV causes degradation of p53, a tumor suppressor gene thus preventing cell cycle arrest or apoptosis. Similarly HPV E7 oncoprotein bind and inactivates the tumor suppressor protein pRB (Retinoblastoma protein), which normally inhibits the progression of cell cycle into S phase.

$P^{16INK4a}$ (henceforth referred to as p16) is a tumor suppressor protein that inhibits cyclin dependant kinase 4 and 6, which phosphorylate the RB protein. A reciprocal relation between p16 and pRB expression has been seen, suggesting the presence of negative feedback loop allowing pRB to limit the concentration of p16. So functional inactivation of pRB by the HPV E7 oncoprotein results in over expression of p16.

p16 protein is detectable immunohistochemically, over expression of it may serve as a surrogate biomarker of HPV infection which makes it useful in evaluating HPV- associated preneoplastic and neoplastic lesions of cervix.

Many literatures have given evidence that p16 may be a very useful marker for preneoplastic, neoplastic squamous lesions and glandular dysplasia of cervix. Moreover, expression of p16 appears to correlate with degree of cervical neoplasia.[10]

Many countries have started vaccination against HPV 16 and 18 and mainly targeted towards adolescent girls. Current vaccines provide excellent efficacy not only against HPV16and 18, but also provide cross protection against non vaccinated types. However HPV vaccines do not protect against all invasive forms of cervical cancer.[11, 12] In developing countries like India, it is necessary to vaccinate all adolescent girls especially in HPV high prevalence area.

Although Pap smear screening test is the easily available test used widely, the gold standard for diagnosis of cervical neoplasm is histopathological examination of cervical biopsy. When we use p16 IHC as an

adjunct to morphological examination, we can recognize the high risk type of HPV infection in those lesions which may progress to high grade lesion.

p16 Immnostaining is a new and cost effective and easily available method which gives valuable information regarding the HPV infection without the need for molecular techniques such as Polymerase chain reaction(PCR), Southern blotting, or Insitu hybridisation (ISH).

Although there are several previous reports on the role of p16 in cervical cancer, there is paucity of them in Indian literature in spite of the fact that cervical cancer is one of the most common cancers among females in India.

This study is an attempt to analyze the association of HPV infection in and around Chengalpattu by using p16 immunostaining in preneoplastic and neoplastic squamous cell lesions of cervix and evaluate its etiological and prognostic benefits as a valuable marker for cervical neoplasm.

AIM AND OBJECTIVES

1. To evaluate the results of expression of p16 in preneoplastic and neoplastic lesions of cervix in order to assess the association of HPV infection in those lesions.

2. To study the pattern of expression of p16 in various histological types of cervical squamous cell lesions by p16 immunohistochemistry.

3. To compare p16 expression in various histological types of cervical squamous cell lesions.

REVIEW OF LITERATURE

Epidemiology

Cervical cancer has become a serious public health issue, being the fourth most common cancer in women worldwide .[1]There is a drastic difference in incidence rate and prevalence of cervical cancer between developed and developing countries. Many developed countries have become successful in reducing the cancer burden over the past six decades through screening programme and other diagnostic workup. Because of the lack of proper health facilities, cervical cancer is leading in developing countries like India.

The age-standardized incidence and mortality rate of cervical cancer in India are 27.0 and 15.2, respectively.[13] An estimated incidence of 1,23,000 cases and 67,000 deaths due to cervical cancer occurred in India in 2012, contributing 23.2% and 25.2% to the global cervical cancer incidence and mortality respectively.[14]It has been estimated that there will be around 205496 new cases and 119097 deaths due to cervical carcinoma by 2020 in India, contributing to 29% and 30% respectively of the global burden of cervical cancer cases and mortality.[15]Cytological screening by pap smear is a very useful test but false positive (15 – 50%) and false negative rates (30%) are high. So histopathological examination of cervical biopsies is regarded as a confirmatory examination in the assessment of cervical neoplasm.[5]

Embryology of Cervix:-

Female reproductive tract organs including uterus, cervix, uterine tubes, and upper part of vagina are developed from mullarian duct, otherwise called as paramesonephric ducts, are a pair of ducts which are present in the intermediate mesoderm. They are formed by invagination of coelomic epithelium. The paramesonephric duct consists of upper vertical part, middle horizontal part and lower vertical part. The upper vertical part lies lateral to the Wolffian duct. Middle horizontal part crosses in front of the Wolffian duct. Both upper and middle part forms the fallopian tube. Lower vertical part fuses with the similar part of the opposite side to form uterovaginal canal in which upper part forms the body of the uterus and cervix, while the lower part forms the upper 4/5 th of vagina. The mullarian ducts meets the endoderm derived urogenital sinus at mullarian tubercle which meet a pair of endodermal sinovaginal bulbs which forms the lower 1/5 th of the vagina.[16]

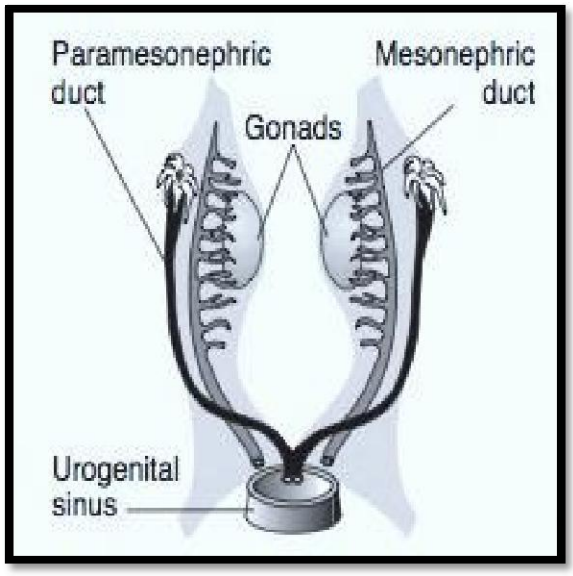


Figure 1: Development of Cervix

Gross Anatomy:-

The uterus is divided into body of the uterus (corpus uteri) which forms the upper two-third, and the cervix (cervix uteri) which forms the lower third. In the adult nulliparous state the cervix tilts forwards relative to the axis of the vagina, called as ante version, and the body of the uterus tilts forward relative to the cervix called as ante flexion. The cervix measures 2.5 cm in length in the adult nulligravida. The lower part of the cervix projects into vagina which divides it into supravaginal and vaginal parts. The parametrium separates the supravaginal portion of the cervix anteriorly from the bladder and also passes laterally between the anterior and posterior layers of the broad ligaments. The vaginal part of the cervix projects into the anterior vaginal wall. The spaces between this part and the vaginal wall are called the vaginal fornices. Through the internal os the upper end of the cervix communicates with the uterus and through external os the lower end of the cervix opens into the vagina. The vaginal portion of the cervix is called as ectocervix and the portion related to the endo cervical canal is known as endo cervix. In nulliparous women, the external os is small and circular, whereas after childbirth become a transverse slit. The cavity of the cervix is fusiform in shape.[17, 18, 19]

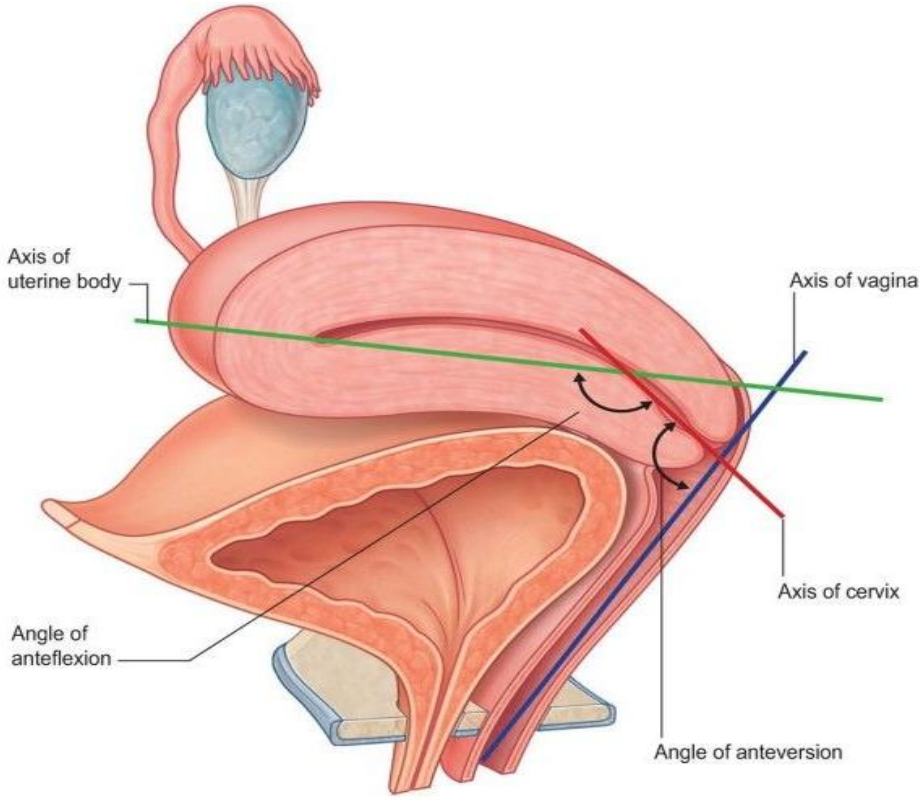


Figure 2: Anatomy of cervix showing angles of antevertion and ante flexion

VASCULAR SUPPLY OF THE CERVIX

Arterial supply

The descending branches of the uterine arteries, reaches the lateral wall of the cervix along the upper margin of the paracervical ligaments and supplies the cervix

Venous drainage

The veins from the cervix form a cervical plexus and run along the lateral border of the uterus. The cervical plexus drains through the uterine, ovarian and vaginal veins into internal iliac veins.

LYMPHATIC DRAINAGE

Lymphatics from the cervix pass laterally in the parametrium and drain into four efferent channels running toward the external iliac and obturator nodes, the hypo gastric and common iliac nodes, the sacral nodes, and the nodes of the posterior wall of the urinary bladder.

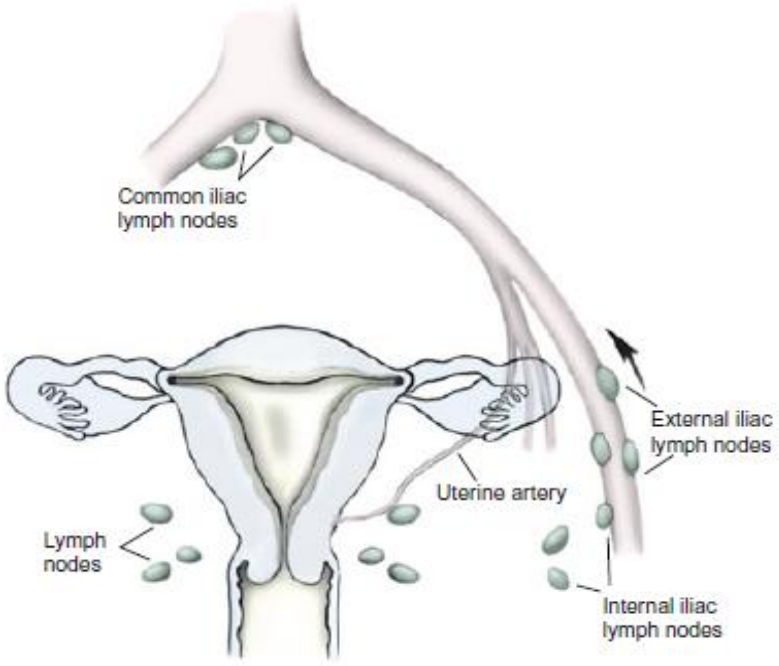


Figure 3:Vascular supply and the lymphatic drainage of the cervix.[20]

NERVE SUPPLY

The cervix is supplied by nerves from the pelvic autonomic system, the superior, middle and inferior hypo gastric plexuses. Pain sensation from the cervix passes along the parasympathetic nerves. The nerve supply is mainly restricted to the endocervix and peripheral deep portion of ectocervix. This causes relative insensitivity of the vaginal portion of the cervix to pain.

LIGAMENTS OF CERVIX

The paracervical ligaments, otherwise called as mackenrodt's ligament and the uterosacral ligaments, attach the supra vaginal portion of the cervix to the second vertebrae through fourth sacral vertebrae, are the greatest sources of fixation and support of the cervix.

HISTOLOGY OF CERVIX

The cervix has a covering epithelium and an underlying stroma. The stroma is an admixture of fibrous, muscular, and elastic tissue. Most of the ectocervix is covered by non keratinizing stratified squamous epithelium, and is composed of three layers of squamous cells: Basal/ parabasal, intermediate, superficial cells. The basal cell layer is one cell thick, with scant cytoplasm and oval nuclei oriented perpendicularly to the basement membrane. The nuclear cytoplasmic ratio decreases progressively from the basal to superficial cells during normal maturation. The parabasal cells are larger than basal cells and have more cytoplasm. The cells in the midzone are called as intermediate cells. The superficial cells have abundant cytoplasm and a pyknotic nuclei than the intermediate cells, and they orient with their longest axis parallel to the basement membrane. The morphological appearance of this various layers varies with age. The cells are become atrophic and exhibit high nuclear cytoplasmic ratio during post menopausal period.

The Endocervix and the endocervical glands are lined by mucinous columnar epithelium. The endocervical glands, represents infoldings of the surface epithelium rather than true glands.[21]

The junction between squamous and mucinous epithelia is known as squamocolumnar junction (SCJ) [22].At birth, SCJ is located on the endocervix which is called as original squamocolumnar junction. Under the influence of estrogen at puberty and pregnancy, the endocervix everts to expose the columnar epithelium, glycogenisation of the epithelium takes place, lactobacilli colonize the epithelium and the PH becomes acidic. These changes stimulate the columnar epithelium to undergo metaplasia and convert into immature squamous and later mature squamous epithelium. With these changes the histologic squamocolumnar junction moves to the external os and this is called as functional or new columnar junction. The area between the original SCJ and the new SCJ is the transformation zone where columnar epithelium is slowly replaced by active metaplasia and this the area where most cervical preneoplastic and neoplastic lesions develop.[23]

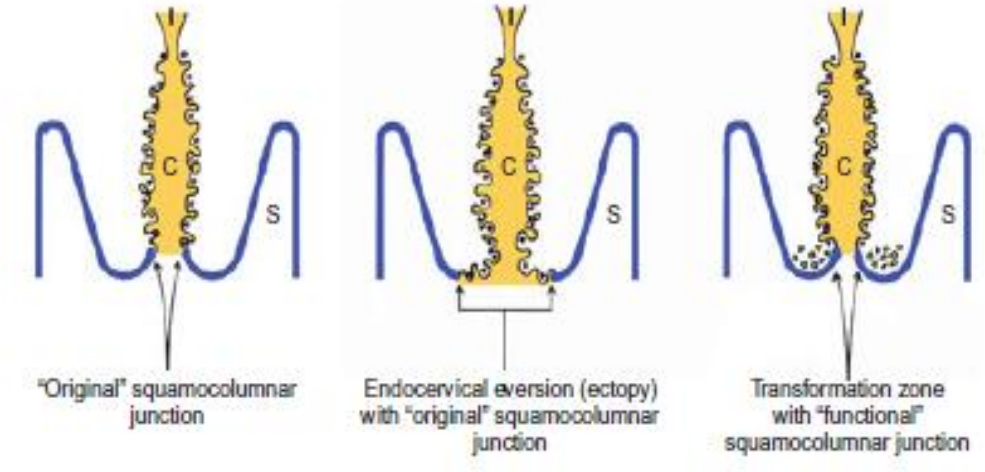


Figure 4 : Schematic diagram of transformation zone.[24]

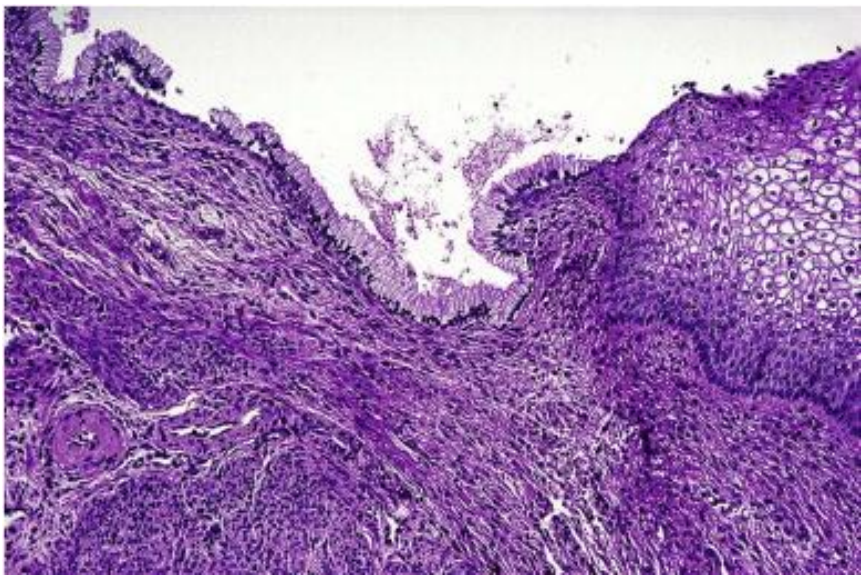


Figure 5 : Transformation zone of uterine cervix.[25]

ETIOLOGY AND PATHOGENESIS

Human papilloma virus

It is now well accepted that cervical cancer is caused by Human papilloma virus. Almost all cervical cancer are directly associated with infectivity with one or more of the oncogenic types of HPV. Approximately 7.9% of women in the general population are estimated to harbor cervical HPV infection at a given time. About 82.5% of invasive cervical cancers are attributed to HPV16 or HPV18. All cervical squamous cell cancers as well as distinct subsets of vulvar, vagina, anal and oral cancers among women and penile anal and oral cancers among men are causally associated with HPV infection.

Epidemiology and Natural history of Human papilloma virus infection

Papilloma viruses are classified as members of papovaviridae family. These are circular double-stranded DNA viruses with approximately 8000 base pairs in length and measures 45 – 55nm in diameter. Its icosahedral capsid composed of 72 capsomers.26Papilloma viruses are epitheliotrophic viruses, means it predominantly infect skin and mucous membrane.[27]

Many lines of evidence have demonstrated that the association between HPV and many types of cervical diseases ranging from the innocuous condyloma acuminatum to fatal invasive squamous cell carcinoma.[28,29,30] At present about 200 types of HPV have been identified and it can be further divided into high- and low-risk types depending on their carcinogenic potential.[31,32,33.]

A large epidemiologic study by Munoz et al observed data from nine countries has identified15 high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82),three probably high risk types(26,53,66) and low risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and cp6108).[34]Of the HPV types infecting the anogenital mucosa, 12 types have been classified as group 1 carcinogens to humans and one is probably of carcinogenic (Group 2A) type. All these 13 HPV types, and also several other possibly carcinogenic types (Group 2B), belong to the same evolutionary branch of the alpha genus in the phylogenetic tree of papillomaviruses (Figure 6). [35, 36, 37]

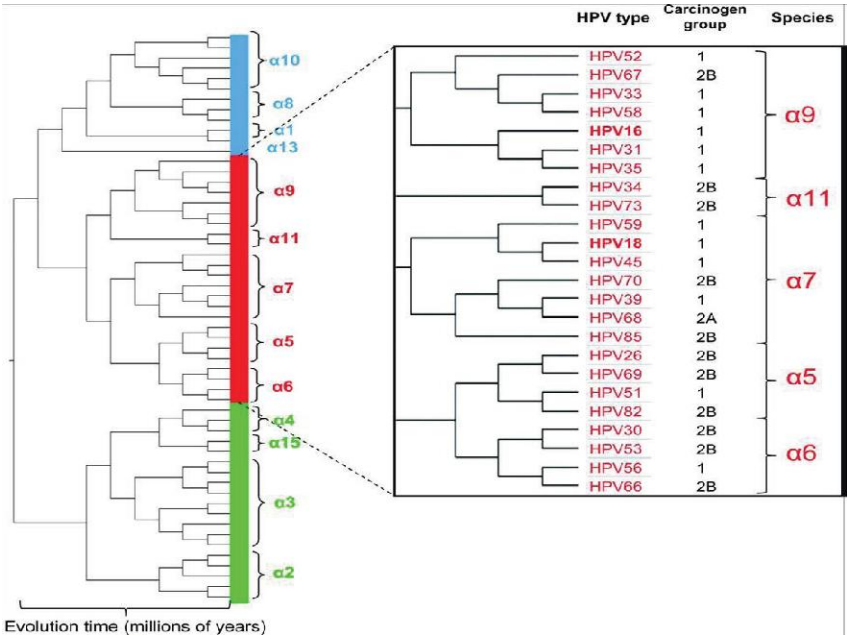


Figure6 : Phylogenetic tree of Alpha – Human papilloma virus.HPV types in red clade are associated with CIN 3 and cervical cancer.HPV types in blue clade cause genital warts. HPV in green clade cause commensal infections.[38]

International Agency for Research Cancer (IARC) classified thirteen anogenital HPV as oncogenic, based on their association with cervical and anogenital cancer which are HPV16,18,31,33,35,39,45,51,52,56,58,59 and 66.[39]In a study by Bosch et al, it was concluded that majority of HPV infections are transient and clear within few months. Persistent high risk HPV infection of the cervical epithelium triggers neoplastic proliferation.[40, 41]

A meta analysis of HPV types in women with LSIL found that HPV was detected in 80% of the LSIL from North America and approximately 70% of LSIL from other regions of the world.[42]

Multiple types of HPV are frequently found in association with LSIL.

TABLE 1: Types of Human papilloma virus with oncogenic risk. [43]

Low oncogenic risk	6, 11, 42, 43, 44, 53
High oncogenic risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66
Unclear oncogenic risk	26, 68, 73, 82

A number of studies have demonstrated that prevalence of HPV 16 from different regions of the world range from 30% to 70%.[42, 44] HPV 16, 18 and 31 have been mostly associated with invasive cervical cancer.[31,45]A large epidemiological study conducted by L loveras et al , evaluate the distribution of HPV types in invasive cervical cancer and showed HPV types 16,18,31,33 and 45 strains cause 85% of the invasive cervical carcinoma worldwide.[46]

Genomic organization of Human papilloma virus

HPV – DNA consist of distinct three different regions. They are early region (ER), late region (LR), upstream regulatory region (URR).The Early region is composed of seven genes,E1 – E7,which play a significant role in viral replication and have oncogenic properties. Late region is composed of two genes, namely L1, the major capsid protein, which can self assemble into virus like particles which are used for the generation of the currently available VLP based HPV vaccines, and L2, the minor capsid protein that is thought to facilitate encapsidation of viral DNA and viral infectivity, The URR is the regulatory region which contains binding sites for both viral and cellular transcription factors.

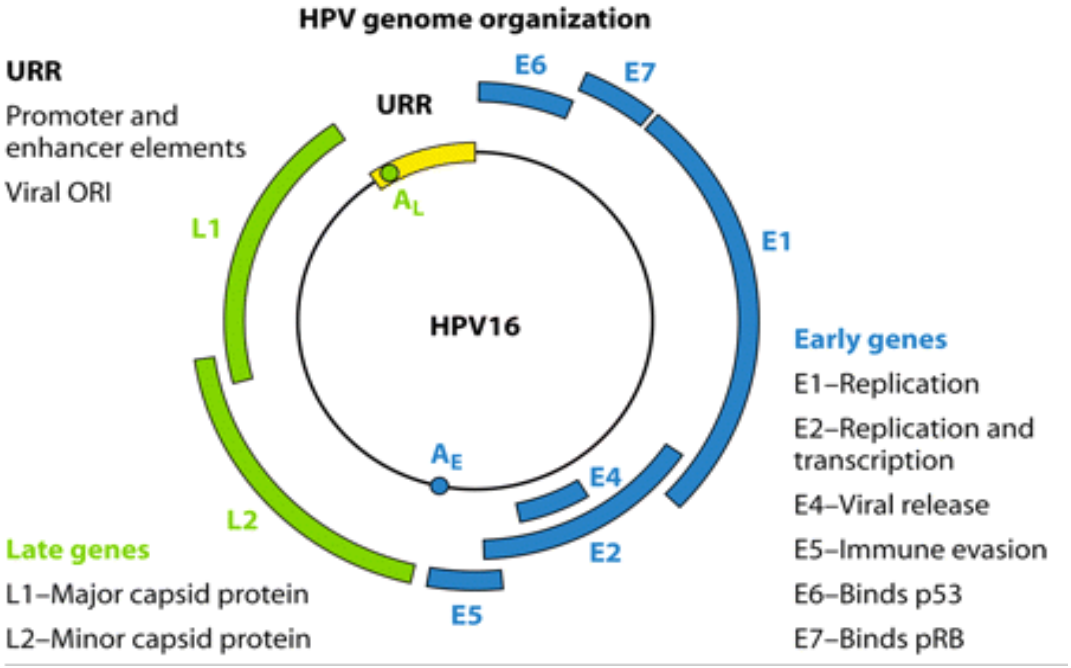


Figure 7 : Genomic organisation of HPV.[47]

In preneoplastic lesions of cervix the HPV DNA is not integrated into the host DNA, rather it is found in circular or episomal form. The episomal HPV produces mostly the E2 protein. This E2 protein encodes for a DNA binding protein that binds to a specific nucleotide motif found in E6 and E7 region. [48,49] E2 regulates the expression of E6 and E7.So that only minimal amount of E6 and E7 is produced. When the episomal form integrates into the host chromosome at E1/E2 region, causing break in this region, results in uncontrolled production and expression of E6 and E7 proteins. This E6 protein forms a complex with p53 tumor suppressor protein leading to degradation of p53.[50]The viral E7 oncoprotein binds to the Retinoblastoma protein (pRb),a tumor suppressor protein and inactivates it. Inactivation of pRb mediates release of transcription factor E2F,which activates the genes necessary for entry of cell into S phase. The accumulation of E2F has been associated with

an increase in INK4A gene transcription, the INK4A gene product, the p16 INK4A protein (p16).

P16, a tumor suppressor gene, located on chromosome 9p21, is belongs to the inhibitors of cyclin dependant kinase (CDK) 4 family. $p^{16INK4a}$is named after its molecular weight (15,845) and its role in inhibiting CDK4.Normally CDK4 and CDK6 binds cyclin D and forms an active protein complex ,which phosphorylate retinoblastoma protein(pRb) .The phosphorylation of pRb induces release of transcription factor E2F from its bound state allowing it to enter into the nucleus. Once in the nucleus E2F promotes the transcription of target genes that are essential for cell cycle progression. p16 binds to the CDK4 and CDK6 and preventing its interaction with cyclin D. This interaction ultimately inhibits the downstream activities of transcription factors, such as E2F and arrest G1-S transition. So inactivation of pRB by E7 causes over expression of p16, because p16 is regulated by negative feedback of pRB.[51] p16 expression is not associated with proliferation, but it is associated with senescence and cell cycle arrest. So p16 expression is not seen in normal cells and actively proliferating cells.

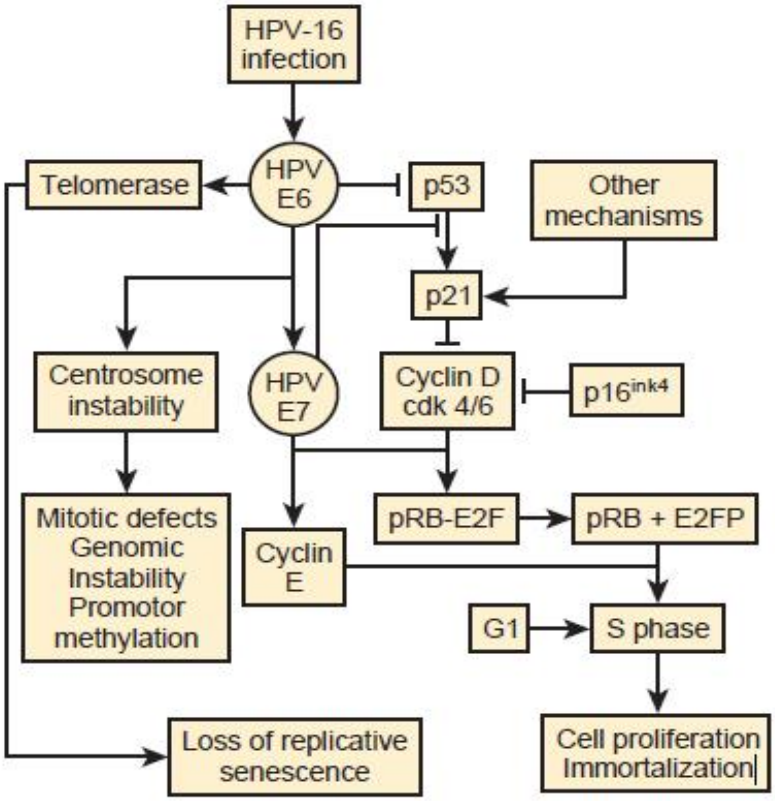


Figure 8 : Molecular basis for cervical neoplasia . This figure shows three pathways involved in HPV related tumorigenesis including alteration in the cell cycle activity induced by E7, up regulation of telomerase via E6 with loss of replicative senescence, and induction of centrosome instability by E6 and E7 with HPV 16 as a model. Progression is also associated with promoter methylation of tumor suppressor gene.52

In a recent study by Iana Leniskova et al concluded that, p16 expression was not seen in normal cervical tissue, but its expression was increased in following frequency : CIN 1 (180/249; 72.3%),CIN2 (212/233; 91.0%). CIN3 (178/181; 98.5%) and invasive carcinoma (131/133; 98.5%).

Life Cycle of Human papilloma virus

HPV initially infects the basal cells or immature squamous cells. It enters into the basal cell layer through defects in the epithelium and remains within the cell in two distinct biological states. In one form HPV continues to remains in the basal cells, without producing virions, this is referred as latent infection. In other form viral DNA replication occurs independently of host chromosomal DNA synthesis, results in large amount of viral DNA formation. Viral DNA replication mainly occurs in intermediate and superficial squamous cells which show the distinct cytological and morphological abnormality, including acanthosis, koilocytosis, multinucleation and nuclear pleomorphism.

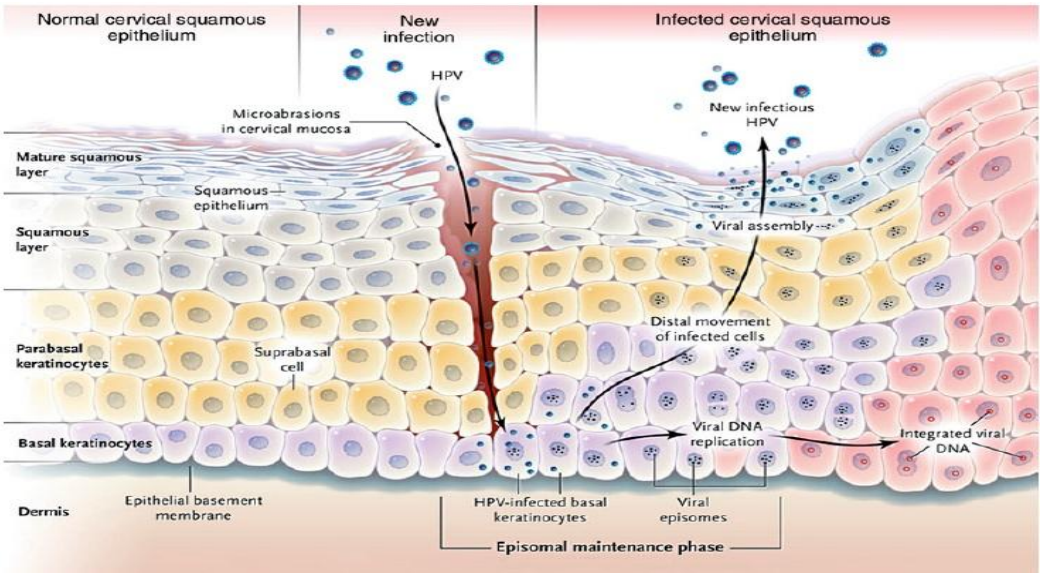


Figure9: Human papilloma virus life cycle [53]

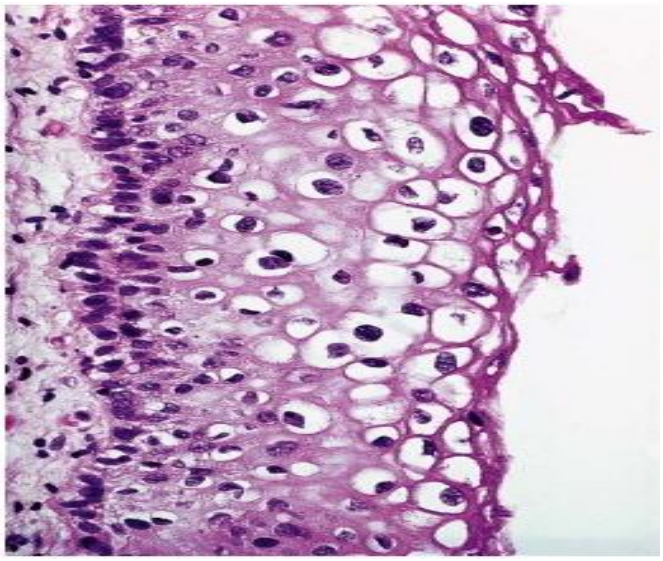


Figure10: Koilocytic changes in the cervical squamous epithelium.[54]

HPV E7 protein is entrapped inside the nucleus. So it is not exposed to the antigen presenting cell, moreover it inhibits the function of interferon α and β. HPV viral proteins which are recognized by dentritic cell are carried to the lymph nodes and also presented to the T cells. The CD4 and CD8 T cells are activated and reach the infected site to destroy the virus. But the Human papilloma virus expose only few viral particles to immune surveillance mechanism, therefore it resides for many years without clinical recognition

Other Risk Factors

Infection with high risk HPV virus is necessary factor for the development of cervical cancer, it is not sufficient for the development of cervical cancer. Because only a small proportion of women exposed to HPV develop cervical cancer, suggest that additional cofactors are necessary in the pathogenesis of cervical neoplasia. These factors may modify the risk in women infected with HPV

Smoking

Szarewski et al concluded that there is a positive association between cigarette smoking and the development of cervical cancer. Some of the studies demonstrated that smoking may be a risk factor only for squamous cell carcinoma, not for adenocarcinoma of cervix.[55, 56]

Oral Contraceptives

A Meta analysis of 28 studies concluded that the relative risk of invasive cervical cancer increased with increasing duration of contraceptive use. There is no associated risk for intraepithelial lesion if it is used less than 5 years but the risk increases to 3 times and 4 times higher if they use oral contraceptives for 5 – 9 yrs and more than 10 years respectively. A large reanalysis of epidemiological studies conducted in more than 50,000 women has confirmed that oral contraceptive use increases the risk of cervical cancer.[57, 58].

Infections other than HPV

Sexually transmitted infections, especially Chlamydial infection is found to be one of a risk factor. Viral infections like Human immunodeficiency virus (HIV) ,Herpes simplex virus -2also plays an important role in the development of cervical neoplasm. The risk of developing cervical cancer is 9.2 times more in women infected with HIV than non infected women.[59]

Immunosuppression

Immunity determines whether the patient is cleared of HPV infection or whether it is persist and progress into malignancy. Studies showed a relative risk of 13.6 for the development of cervical carcinoma in situ in renal transplant recipients compared to women in the general population.[60] Because of the immunosupression in HIV status ,they are more prone to develop cervical cancer than women in general population.

Sexually associated factors

Multiple sexual partners, early age at first intercourse, early marriage, male sexual behavior, concurrent penile cancer in males are also an important sexually associated factors in pathogenesis of cervical neoplasm.

Other factors

There are various other risk factors associated with development of cervical cancer. They are dietary deficiency, early age at first pregnancy, multiparity, low socioeconomic class. [61, 62]

Table2: Risk factors for cervical cancer: HPV infection vs. persistence and Malignant transformation

Risk factor	HPV infection	HPV persistence And transformation
Multiple sex partners	+	n.e.
Partner's multiple partners	+	n.e
Poor hygiene	+	n.e
Absence of male circumcision	+	+
Immunodeficiency, HIV	+	+
High parity	n.e	+
Oral contraceptives	n.e	+
Smoking	n.e	+
STDs other than HPV	n.e	+
Poor nutritional status	n.e	+

STDs = Sexually transmitted diseases (especially C, trachomatis).

n.e = No evidence for being a risk factor at this time.

Studies in transgenic K14E7 mouse models showed that estrogen receptor is required for the initiation and maintenance of cervical cancer .[63]

Another study demonstrated that HPV oncogenes promote squamous cell carcinoma by an additional mechanism of micro RNAs. [64, 65]

TABLE 3:WHO Histological classification of tumors of the uterine cervix

I	**EPITHELIAL TUMORS :-**	
1.	Squamous Tumours and precursors	
1A	Squamous cell carcinoma, not otherwise specified	➢ Keratinizing ➢ Non -Keratinizing ➢ Basaloid ➢ Verrucous ➢ Warty ➢ Papillary ➢ Lymphoepithelioma – like ➢ Squamotransitional
1B.	Early Invasive squamous Cell Carcinoma	
1C.	Squamous intraepithelial neoplasia	➢ Cervical intraepithelial neoplasia ➢ Squamous Cell Carcinoma insitu
1D.	Benign Squamous Cell lesions	➢ Condyloma acuminatum ➢ Squamous Papilloma ➢ Fibro Epithelial polyp
II	Glandular tumours and precursors	
III	Other epithelial tumours	
IV	Mesenchymal tumours and tumour like conditions	
V	Mixed epithelial and mesenchymal tumours	
VI	Melanocytic tumours	
VII	Miscellaneous tumours	
VIII	Lymphoid and haematopoietic tumours	
IX	Secondary tumours	

PRENEOPLASTIC LESIONS OF CERVIX

Natural history of cervical intraepithelial neoplasia

Genital HPV lesions are more common among women in reproductive age group, but most of them are asymptomatic. On average 50% of the infections cleared within 8 months and 90% of the HPV infections cleared within two years. Persistent high risk HPV type infection is the major risk factor for the development of both squamous cell carcinoma and adenocarcinoma of the cervix.[33]However, the natural history of squamous cell carcinoma of cervix is well understood than adenocarcinoma.[66] The development of cervical cancer is a multistep process in which precancerous lesions persist, progress and regress overtime except the last step leading to invasive lesion is not reversible.

The precancerous lesions of cervix are usually described as cervical intra epithelial neoplasia. [67]The cellular changes in precancerous lesions of cervix involves nuclear atypia, increased nuclear cytoplasmic ratio, mitotic activity limited to the surface epithelium and do not extend beyond the base ment membrane. There are different classification systems for cervical precursor lesions which are used interchangeably overtime. Older Papanicolaou classification used the term as ‘atypical cells with abnormal features’. Another classification system grouped the lesions into mild, moderate, severe dysplasia and carcinoma in situ. The cellular changes are limited to lower one third of epithelium in mild dysplasia, extend to middle one third in moderate dysplasia and to upper one- third in severe dysplasia. Full thickness involvement is called carcinoma in situ. This was followed by

cervical intraepithelial neoplasia (CIN) classification. In this classification mild dysplasia were termed CIN I, moderate dysplasia CIN II, and severe dysplasia and carcinoma insitu termed CIN III. This three tier classification system has been recently simplified into two tier system , with CIN I , condyloma acuminatum, Exophytic condylomas and squamous papilloma are coming under low grade squamous intraepithelial lesion (LSIL) and CIN II ,CIN III and carcinoma insitu combined into one category called as high grade squamous intraepithelial lesion (HSIL).[68]

Table4 : Classification of HPV associated intraepithelial lesions of cervix.[69]

Term	HPV risk category	Comparison of classification systems		
		Two-tiered CIN	Dysplasia/CIS	SIL
Exophytic condyloma	Low risk	_____	_____	LGSIL
Squamous papilloma	Low risk	_____	_____	LGSIL
Flat condyloma	Low and high risk	_____	_____	LGSIL
CIN 1	Low and high risk	Low grade CIN	Mild dysplasia	LGSIL
CIN 2	High risk	High grade CIN	Moderate dysplasia	HGSIL
CIN 3	High risk	High grade CIN	Severe dysplasia/ CIS	HGSIL

CIN = Cervical intraepithelial neoplasia
SIL = Squamous intraepithelial lesion
CIS = Carcinoma in situ
LG = Low grade
HG = High grade

Generally more than 80% of LSIL lesions and 100% of HSIL lesions are associated with high risk HPV types. A study conducted by Moscicki et al ,showed in terms of CIN lesions of any grade, up to 90% regress spontaneously in women aged 13 to 22 years , whereas among women 34 years and older, the estimated risk of regression is only 40%. In Boyes et al study, 77% of the most severe preinvasive lesions, carcinoma in situ, regressed spontaneously among women younger than 40 years-old whereas the estimated rate of regression is 61% among women aged 40 and older. McCredie et al undertook study in New Zeeland reported that 20% of women with untreated CIN3 lesions developed cancer cervix within 10 years and 31% within30years. Generally, the median time from initial exposure to HPV to the development of carcinoma in situ is at least 7 to 12 years.[70]

Table5: Natural history of CIN depend upon lesion grade.[71]

	% Regression	% Persist	Progress to CIS
CIN 1	57	32	11
CIN 2	43	35	22
CIN 3	32	56	12

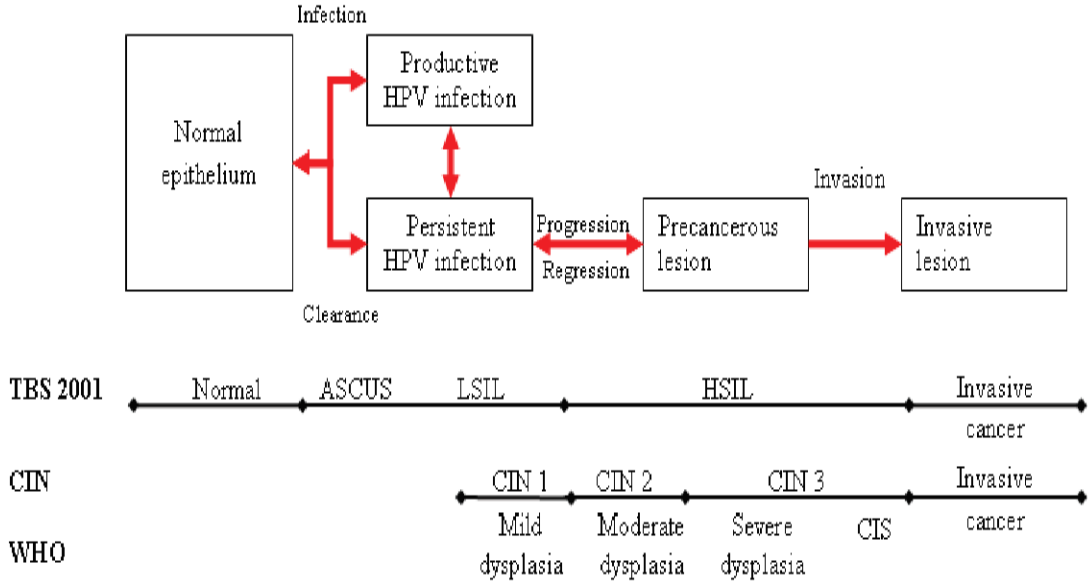


Figure 11 : Progression Model of Cervical Carcinoma

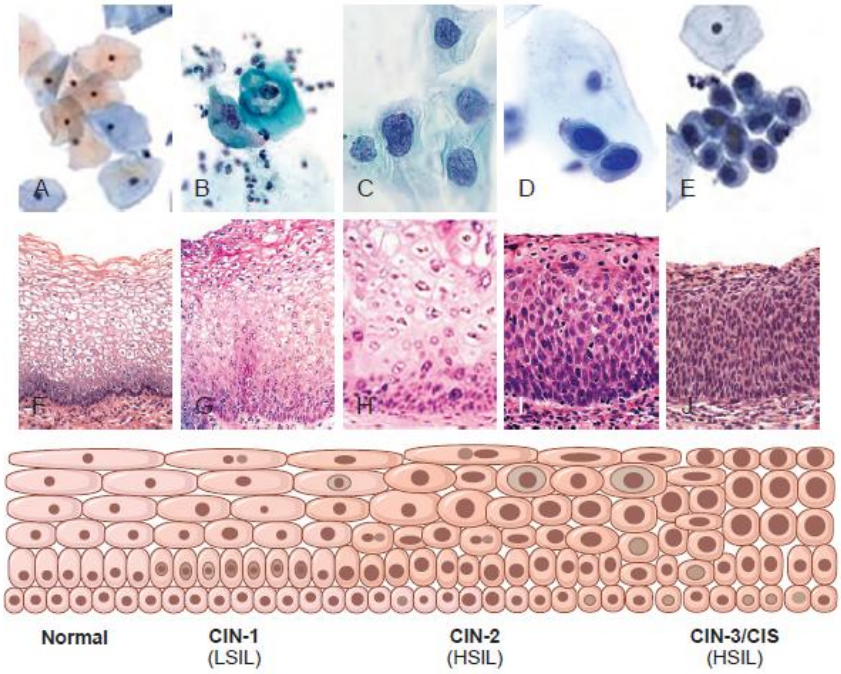


Figure 12 : A classic schematic diagram of cervical intraepithelial neoplasia (lower) defines the cytopathologic (A -E) and histopathologic (F-J) transitions from normal to LSIL (CIN-I) toHSIL (CIN2 & 3).[52]

Low grade squamous intraepithelial lesion (LSIL)

Normal squamous cell nuclei become smaller when they mature and move towards the surface. In low grade squamous intraepithelial lesion also cells are mature squamous cells with polygonal shape, but nuclei are enlarged at least 3 – 4 times, that of normal intermediate cell nucleus. The cells become smaller and the nuclei become smaller and pyknotic, irregular nuclear contour sometimes with bi/multinucleation and cytoplasmic halos when HPV changes are evident. These pyknotic nuclei may also exhibit features like increased size that of the normal superficial squamous cell and a mild nuclear atypia, fine to coarsely granular and evenly distributed chromatin, hyperchromasia. Bi nucleated cells are present in 90% of the LSIL and when they surrounded by cytoplasmic halo are termed koilocytes.[52]

Condyloma acuminatum, Immature condyloma / squamous papilloma, Flat condyloma are the three morphological subsets of LSIL.

Moreover, LSIL do not progress directly to invasive cervical cancer, because most cases regress spontaneously, only few cases progress to HSIL. So LSIL is not treated like a premalignant condition.

High grade squamous intra epithelial lesion

High grade squamous intraepithelial lesion is characterized by presence of atypical cells with nuclear pleomorphism, irregular nuclear contours and coarse chromatin along with loss of cell polarity, increased mitotic index with

mitosis in the upper half of the epithelium and abnormal mitotic figures. If these abnormalities involve one third to two third of mucosal thickness, it is said to be Cervical intra epithelial lesion 2(CIN2),and more than two- thirds ,it is called as cervical intraepithelial lesion 3(CIN3).Apart from the above, nuclear cytoplasmic ratio is considered as a important criterion to diagnosis of HSIL. The nuclei in LSIL can be markedly enlarged and pleomorphic but have low nuclear cytoplasmic ratio, whereas the nuclei in HSIL are more uniform but with irregular nuclear contours and high nuclear cytoplasmic ratio. LSIL involves superficial layers of the mucosa, whereas in HSIL the atypical cells extend upwards from the basal layer to at least one third of the mucosal thickness.

Early invasive (micro invasive) squamous cell carcinoma

It is defined as stromal invasion of malignant squamous cells by less than or equal to 3mm in depth and 7mm in length. But assessment of this early stromal invasion is very difficult. The criteria for the diagnosis of early invasion include

1. Desmoplastic response in the adjacent stroma

2. conspicuous maturation of malignant squamous epithelium

3. Blurring of epithelial stromal interface

4. Loss of polarity of nuclei at the epithelial stromal interface.[72]

Invasive squamous cell carcinoma

The most common malignant tumor of female genital tract in both developed as well as developing countries is invasive squamous cell carcinoma of cervix. The role of HPV in the pathogenesis of all squamous cell carcinoma has become obvious, which was discovered by Harald zur Hausen, for that he was awarded the Noble prize in 2008.

Invasive squamous cell carcinoma of cervix can be classified by grade (well differentiated, moderately differentiated and poorly differentiated) and/or by morphology (Large cell keratinizing, Large cell non keratinizing and small cell non keratinizing) . WHO now recommend two tiered classification as keratinizing and non keratinizing tumors to avoid confusion with small cell carcinoma. But the grade and type have not found to be prognostically significant, instead, the depth of invasion, size, lymphatic or vascular invasion are the important prognostic variables.

MORPHOLOGY

Gross

Grossly, invasive cervical carcinoma may be polypoid or fungating or deeply infiltrative. Infiltrative carcinomas invade adjacent structures more commonly than polypoidal type.

MICROSCOPY

Keratinizing

These tumors are considered as well differentiated tumor , shows conspicuous evidence of keratinization in the form of keratin pearls, keratohyaline granules, individual keratinized cells and nests of squamous cell with central keratinization.The nuclei are large, hyper chromatic with coarse chromatin. Mitotic figures are not commonly seen.

Non keratinizing

These tumors are composed of large squamous cells which are polygonal in shape with eosinophilic cytoplasm but lack the evidence of keratin pearls. Cellular and nuclear pleomorphism is more obvious with numerous mitotic figures. Non keratinizing carcinomas are typically moderately differentiated.

Basaliod

Some invasive squamous cell carcinoma have nests of basal type squamous cells having scant eosinophilic cytoplasm and peripheral palisading of nuclei with variable amount of squamous differentiation. These carcinomas are typically poorly differentiated.

OTHER RARE VARIANTS OF SQUAMOUS CELL CARCINOMA

Verrucous

These tumors are exophytic and composed of broad based papillae lined by squamous epithelium with little or no atypia. These tumors have pushing margin.

Warty or condylomatous

These tumors are exophytic squamous cell carcinoma that have koilocytic surface epithelial changes characteristic of HPV infection.

Papillary

This type of tumor is characterized by a papillary growth pattern and it is subdivided into three histological subtypes.

1. Papillary undifferentiated carcinoma: - In this carcinoma, the tumor cells lining the papillae do not show histological evidence of specific type of differentiation.

2. Papillary transitional cell carcinoma: - Has a similar histologic appearance to lesions that occur in the urinary tract.

3. Papillary squamotransitional carcinoma:-which has a combination of transitional and squamous features.

Lymphoepithelial – like

It resembles undifferentiated nasopharyngeal carcinoma and consists of poorly defined aggregates of nonkeratinizing tumor cells with large vesicular nuclei, prominent nucleoli and moderate amount of eosinophilic cytoplasm, syncytial appearance and heavy lymphocytic infiltration.[73]

DIAGNOSIS AND AIDS TO DIAGNOSIS

Diagnosis is done by

- History
- Physical examination
- Investigations

History

Any women of reproductive age presenting with abnormal uterine bleeding, post coital bleeding, white discharge, pelvic pain, mass per vaginum, urinary or bowel symptoms should suggest the possibility of cervical cancer.

Physical Examination

- General examination – Cachexia, pallor , supraclavicular and inquinal nodes
- Systemic examination
- Abdominal examination – Ascites, Enlarged uterus, Hepatomegaly
- Speculum examination – Growth on the cervix (Type of growth, Bleeds on touch, Fixity).

Investigations

- Pap smear
- HPV testing
- Cervical biopsy
- Cystoscopy / proctoscopy/IVP
- USG/CT / MRI
- Complete blood count, liver function test, Renal function test. [74]

PREVENTION AND EARLY DETECTION OF CERVICAL CANCER

Prevention of cervical cancer consists of creating awareness about the risk factors through health education, promoting practice of safe sex, use of condoms to prevent STDs, lifestyle modification, screening and early treatment of premalignant lesions and HPV vaccines.

Cervical cancer screening

Several screening methods are available, but cytology (Pap smear) is the most widely used method.

Methods used for cervical cancer screening

- Cytology

  Conventional cytology (Pap smear)

  Liquid based cytology (LBC)

  Manual interpretation

Automated screening

- Visual inspection after acetic acid(VIA)
- Visual inspection after acetic acid with magnification(VIAM)
- Visual inspection after Lugol's iodine(VILI)
- Cervical biopsy
- HPV testing
- Investigational strategies

  Polar probe

  Laser – induced fluorescence

HPV detection techniques

1. Immunohistochemistry
2. Southern Blot
3. Dot Blot assays
4. In situ Hybridization
5. Hybrid capture 2 assay (HC2)
6. Polymerase chain reaction
7. HPV genotyping
8. Immunocytochemical detection of L1 capsid protein

The only test presently approved by U.S.Food and Drug Administration is the Hybrid capture 2 assay (HC2) test..But it is not widely available. Among the above mentioned tests , immunohistochemistry, In situ Hybridization,PCR are the most commonly used methods.

CERVICAL CYTOLOGY

The abnormal cells of cervical neoplastic exfoliate which can be collected by scraping the cervix and staining the smear. Based on the severity of abnormality, it is possible to diagnose cervical pre-neoplastic and neoplastic lesions . This method of screening was first introduced by Papanicolaou and is known as Pap test or Pap smear.

Screening guidelines

- Begin at age 21
- Screen every 2 years till age 30
- Screen every 3 years from age 30 if

  Three consecutive negative smears

  No CIN II or III / HIV infection in the past

  Not immunocompromised

  No DES exposure in utero
- Stop screening at 65 – 70 if previous three smears negative, except when performed for CIN II /III

- No screening after hysterectomy
- Conventional cytology or LBC can be used
- For women > 30 years ,combined cytology and HPV testing recommended

Table 6: The Bethesda System of Cytologic Classification (2001)

Specimen type

Indicate conventional smear (Pap smear) versus liquid based versus other

Specimen adequacy

- Satisfactory for evaluation
- Unsatisfactory for evaluation (specify reason)
- Specimen rejected / not processed (specify reason)
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

General categorization (optional)

- Negative for intraepithelial lesion or malignancy
- Epithelial cell abnormality. (See Interpretation/result [specify
- 'squamous' or 'glandular' as appropriate))
- Other: See Interpretation/result (e.g., endometrial cells in a woman over 40 years of age)

Automated review (specify)

Ancillary testing (specify)

Interpretation/result

Negative for intraepithelial lesion or malignancy

- Organisms (specify)
- Other non-neoplastic findings (optional to report; list not inclusive)
- Other (specify)

Epithelial cell abnormalities

- Squamous cell
- Atypical squamous cells of undetermined significance (ASCUS) cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) encompassing: HPV/miki dysplasia/CIN-1
- High-grade squamous intraepithelial lesion (HSIL) encompassing: moderate and severe dysplasia, CIS/CIN-2 and CIN-3 (with features suspicious for invasion (if invasion is suspected)

Squamous cell carcinoma

MANAGEMENT OF ABNORMAL SMEARS

The risk of developing CIN II or III after ASC-US is approximately 5-10%.So aggressive management is not required. HSIL is found in 25% of women with ASC-H, therefore immediate colposcopy is recommended. Colposcopy is usually the course of action in LSIL since CIN II or III may be found in 15 – 20%.All women with HSIL must have immediate colposcopy evaluation.

COLPOSCOPY

Colposcopy is performed in all women with abnormal cytology. Colposcopy helps in localization of the lesion and taking a directed cervical biopsy

MANAGEMENT OF LSIL

Since rate of progression of LSIL to invasive cancer is low ,aggressive management is not indicated. Repeat smear 6 - 12 months later.HPV DNA testing may be performed at 12 months, and if negative, routine screening is recommended.

MANAGEMENT OF HSIL

HSIL lesions are treated by excision or ablation.[76]

Treatment modalities

- Ablative procedures

- Thermo ablation

  - Cryotherapy

- Carbondioxide laser

  - Excisional procedures

    - Loop electroexcision procedures

    - Cold knife conisation

    - Carbon dioxide laser

  - Hysterectomy

Table 7:TNM and FIGO Classification of carcinoma of the uterine cervix

TNM Classification T- Primary Tumour

TNM Categories	**FIGO Stages**	
Tx		Pnmary tumour cannot be assessed
To		No evidence of pnmary tumour
Tis	0	Carcinoma in situ, (preinvasive carcinoma)
T1	1	Cervical carcinoma confined to uterus (extension to corpus should be disregarded)
T1a	IA	Invasive carcinoma diagnosed only by microscopy. All macroscopically visible lesions even with superficial invasion are Tlb/ Stage 1B
T1a1	IA1	Stromal invasion no greater than 3.0 mm in depth and 7.0 mm or less in horizontal spread
T1a2	IA2	Stromal invasion more than 3.00 mm and not more than 5.0 mm with a horizontal spread 7.0 mm or less
T1b	1B	Clinically visible lesion confined to the cervix or microscopic lesion greater than Tla2/1A2
T1b1	1B1	Chnical visible lesion 4.0 cm or less in greatest Dimension.
T1b2	1B2	Clinically Visible lesion more than 4 cm in greatest Dimension.
T2	II	Tumour invades beyond uterus but not to pelvic wall or to lower third of the vagina.
T2a	IIA	Without parametrial invastion
T2b	IIB	With parametrial invasion
T3	III	Tumour extends to pelvic wall, involves lower third of vagina, or causes hydronephrosis or non-functioning kidney

T3a	IIIA	Tumour involves lower third of Vagina no extension to pelvic wall.
T3b	IIIB	Tumour extends to pelvic wall or causes hydronephrosis or non-functioning kidney
T4	IVA	Tumour invades mucosa of the bladder or rectum or extend beyond true pelvis.

N- Regional Lymph Nodes

NX		Regional lymphnodes can not be assessed
N0		No regional lymphnode metastasis
N1		Regional lymphnode metastasis

M - Distant metastasis

M1	IVB	Distant metastasis

TREATMENT OF INVASIVE CERVICAL CARCINOMA

Treatment of invasive squamous cell carcinoma involves surgery, Radiotherapy, Chemotherapy depending on the extent of tumor involvement and general condition of the patient. FIGO stage IA1 tumors can be treated with simple hysterectomy or large loop excision in women who wish to preserve fertility.[77] Patients with FIGO Stage IA2 tumors and above are treated with modified radical hysterectomy and regional lymph node dissection. Patients with FIGO stage IB to IIA tumors can be treated with radical hysterectomy or with radiation therapy.[78] FIGO IIB to IVB tumors are treated with radiation therapy and concurrent chemotherapy .In case of post irradiation relapse of cervical carcinoma, pelvic exentration should be considered.[79]

- Stage IA1 – Conisation or simple hysterectomy
- Stage IA2 – Modified radical hysterectomy with pelvic lymphadenectomy
- Stage IB1 – Radical hysterectomy with pelvic lymphadenectomy(or) chemo radiation
- Stage IB2 – Radical hysterectomy with pelvic lymphadenectomy and postoperative radiation
- Stage III – Chemo radiation
- Stage IVA – Chemo radiation
- Stage IVB – Palliative radiation(or) palliative chemotherapy

SPREAD AND METASTASIS

Direct spread

The squamous cell carcinoma of cervix spreads to uterus, vagina, parametrium, Utrosacral ligaments and lower urinary tract by direct extension.

Lymphatic spread

Lymph node metastasis proceeds in a sequential fashion. The paracervical, hypogastric, obturator and external iliac groups are the first station, and the second station is represented by the sacral, common iliac, aortic and inguinal groups. The nodal involvement is directly related to the stage of the disease.[80]

Hematogenous spread

Distance metastasis may be seen in lungs (9%),bones (4%),liver and other structures.[81, 82]

Prognosis

The prognosis of invasive squamous cell carcinoma of cervix is related to the following parameters. [54]

1. Clinical staging :- most important prognostic determinator
2. Nodal status, size and number of positive nodes
3. Depth of invasion

4. Endometrial extension, parametrial involvement and blood vessel invasion

5. Microscopic type

6. Microscopic grade

7. Tumor associated tissue eosiniphilia;-

   This feature is regarded as good prognostic sign.

8. Cell proliferation index

9. HPV:- Lombard I, Vincent – Salomon et al study shows ,patients with intermediate risk HPV ,the 5 year disease free survival was 100%, 58% for patients with HPV16 positive tumors and 38% for patients with HPV18 positive tumors. Also absence of detection of HPV in the tumor cells indicate poor prognostic sign.

10. Expression of HER2/neu, RAS oncogene, Tn antigen, allelic loss of chromosome1,stromal infiltration by S-100 protein positive langerhans cells are associated with poor prognosis.

MARKERS COMMONLY USED IN PRENEOPLASTIC AND NEOPLASTIC SQUAMOUS LESIONS OF CERVIX:-

$p^{16INK4a}$:-

Many literatures have demonstrated that $p^{16INK4a}$ may be a useful marker for preneoplastic and neoplastic squamous cell lesions of cervix.$p^{16ink4a}$

is a cellular correlate of the increased expression of the viral oncoprotein E7 which distrupt the tumor suppressor protein, pRb. The disturbance of the Rb pathway leads to a compensatory over expression of p16 through a negative feedback loop.[83]Furthermore , p16 over expression is correlate well with the degree of cervical neoplasia.[10,84] The role of p16 INK4A has been reviewed in many previous articles[85,86] These reviews showed significant heterogeneity in methods used for defining p16 positivity, so there is wide range in the sensitivity(59% – 96%) and the specificity (41% – 96%) reflecting heterogeneity in interpretation and analyzed population. Over expression of p16 is seen only when HPV has integrated into the genome of the host and this does not occur in low risk types of HPV.p16 is generally a nuclear protein, but overproduction of it force it into the cytoplasm.

Murphy et al in 2005 analyzed and compared the expression patterns of three potential biomarkers p16, CDC6 and MCM5.Among three markers they found that the p16 as a reliable marker of cervical dysplasia and its expression was closely linked with high risk HPV infection.

In some of the studies, p16 was used as a prognostic marker. A four year follow up study conducted by Negri et al in 2004 assessed the role of p16 in predicting the progression of CIN I to CIN III. They concluded that p16 was seen in low grade lesions of cervix which may undergo spontaneous regression, but cases with diffuse p16 staining had a significantly higher chance to progress to high grade lesion than p16 negative cases.(109) A 2 year follow up study by omori et al in 2007 observed that CIN 2 lesions with diffuse p16 staining had a higher risk of progression to CIN 3.[87]

MIB – 1 as a proliferation marker in cervical neoplasia :-

MIB -1 (Molecular immunology Borstel) is an important proliferation marker for CIN. Gerdes et al in 1990 demonstrated that MIB -1 antibody detects Ki – 67 antigen in the G1,S, G2 and M phase, but it is absent in G0 phase. Ki – 67 is an antigen expressed in proliferating cells. Several studies have demonstrated that this antibody may be a useful marker of proliferative activity of preneoplastic and neoplastic lesions of cervix.

Keating et al in 2001 analyzed the staining patterns of p16 and MIB- 1 in normal, reactive epithelial changes, LSIL and HSIL. Expression of these markers are closely linked to the grade of the SIL .Positive scores for Ki -67 and p16 were seen in 68.4% and 100% of LSIL and 94.7% and 100% of HSIL respectively.

Markers of aberrant S-phase induction :-

The cell cycle activation mediated by HPV oncogene in transforming infections is characterized by aberrant S – phase induction. Topoisomerase IIA (TOP2A) and minichromosome maintenance protein 2 (MCM2) are the two proteins detected by an assay which is commercially available (proEx C) . There are only few literatures that studied these markers and that too on minimal samples and their result showed sensitivity of 67% - 99% and specificity of 85%. [88]

Other biomarkers undergoing clinical validation :-

Other cellular makers such as CK13 and CK14[89], MCM5 and CDC6[90], Survivin[91]and CEA.[92], Telomerase /TERC and Ki – 67 have also been evaluated in various stages of development. But most of them are marked by non-uniformity in determination of end points and limited sample sizes. Other viral markers like HPV L1 capsid protein[93]and E6 oncoprotein[94]detection are also under study, but further evidence is needed to confirm their utility.

Among all of the immunomarkers, p16 INK4A expression is considered as a valuable and cost effective marker for cervical intraepithelial neoplasia and squamous cell carcinoma of cervix.[95]According to different studies, immunohistochemistry (IHC) has a sensitivity of 52-87% for the detection of HPV.[96]

MATERIALS AND METHODS

Study place:

Departement of Pathology, Chengalpattu Medical College, Chengalpattu

Study design:

The present cross-sectional study was a prospective study conducted in the Department of Pathology during the period of June 2014 to August 2015. Ethical clearance for the study was obtained from the Ethics Committee of Chengalpattu medical college, Chengalpattu.

A total sample of 60 cases, including both preneoplastic and neoplastic squamous cell lesions of cervix, were analyzed during the period of June 2014 to August 2015

Inclusion criteria

Tissue blocks of patients who are diagnosed as CIN (Cervical Intraepithelial neoplasia) I, II, III and squamous cell carcinoma of cervixon histopathological examination were included in this study.

Exclusion criteria:

Tissue blocks of squamous cell carcinoma patients who underwent Radiotherapy or Chemotherapy were excluded in this study.

Methodology and Technique Used:

Materials needed:

- Hematoxylin & Eosin stain
- p16 ^{INK4a} immunohistochemical marker kit
- Formalin fixed & paraffin embedded blocks which were reported as CIN I, II, III and Squamous Cell Carcinoma of Cervix

Methods :

- All blocks and slides of 60 patients in whom Cervix biopsy was reported as CIN I, II, III and Squamous Cell Carcinoma of Cervix as per standard protocol were taken for study.
- Immunohistochemistry was performed on the 60 study sections.
- 4-micrometer thin sections were cut & placed on charged slides and incubated at 60 – 70 degree Celsius for 1 hour.
- Sections were deparaffinized in xylene for 15 minutes x 2 changes and rehydrated through graded alcohols as follows:-
  - Absolute alcohol – Two changes for 5 minutes each.
  - 90% alcohol - for 5 minutes
  - 70% alcohol - for 5 minutes
- Then sections were washed in distilled water two changes for 2 minutes each.

- Antigen retrieval was carried out at 150 degree Celsius in citrate buffer, (pH = 9) for 15 min and washed in Tris Buffer Solution buffer solution for 20 minutes.

- The slides were cooled to room temperature and washed in distilled water for 2 changes 5 minutes each.

- Then washed in Tris Buffer Solution for 2 minutes.

- By adding 1% hydrogen peroxide on the sections and kept for 5 minutes the endogenous peroxidase activity was blocked.

- The slide was washed in buffer solution for 2 minutes each.

- Primary antibody ($p^{16INK4a}$– clone G175 – 405 – Mouse monoclonal antibody) was added and kept for 30 minutes at room temperature then washed in buffer solution for two minutes, two times each.

- Secondary antibody (Polyexcel Target binder reagent) was applied and kept for 15 minutes then washed in two changes of buffer 2 minutes each , followed by incubation with Horse radish peroxidase for 15 minutes .

- Colour was developed by incubating the sections with diamino benzidene for 5 minutes then washed in distilled water & sections were counter stained with haematoxylin for 2 seconds.

- The slides were washed in running tap water for 3 minutes. The slides were air dried, cleared with xylene and mounted with DPX.

- For positive control – Histological section of Squamous Cell Carcinoma Cervix with known P16 positivity was included in each batch of staining.

- For negative control – Phosphate buffer solution was used instead of primary antibody.

- p16, immunostained sections were reviewed. Chestnut brown colour in the nucleus and/or cytoplasm was considered as immuno positivity.

- Grading was performed for each case by counting the number of positive cells in different epithelial clusters as Grade 1(1 – 5 %), Grade2(5–25%) and Grade 3(>25%,), based on the number of positive cells.

DATA COLLECTION:-

All the 60 histopathological slides were reviewed and the diagnosis was categorized as below:-

- Cervical Intraepithelial neoplasia - I (CIN-I)
- Cervical Intraepithelial neoplasia -II (CIN-II)
- Cervical Intraepithelial neoplasia - III (CIN-III)
- Early invasive squamous cell carcinoma of cervix.
- Large cell keratinizing Squamous cell carcinoma of cervix.
- Large cell non keratinizing Squamous cell carcinoma of cervix.
- Small cell non keratinizing Squamous cell carcinoma of cervix.

Interpretation of $p^{16INK4a}$ staining

For all the above 60 cases p16 expression was expressed. Two parameters were evaluated in p16 expression.

1. Percentage of p16 positive cells

2. Reaction intensity of p16 immunostaining

The percentage of $p^{16ink4a}$ positivity was graded by determining the percentage of $p^{16ink4a}$ imunoreactive cells that is percentage of cells with brown nuclear and/ or cytoplasmic reactivity. The grading for p16 expression was graded as below.

Negative

- When no cells stained
- Weak Cytoplasmic staining

Positive

- Grade 1 :-positive cells >0 – 5%
- Grade 2 :-positive cells >5 – 25%
- Grade 3 :-positive cells >25%

P16 intensity of reaction was scored as:-

- Negative
- Weak
- Moderate

- Strong

Statistical analysis:

Data obtained was coded and entered into Microsoft excel spread sheet (Annexure II). The data was analyzed by using SPSS version 16. Continuous data was expressed as mean and median. Correlation between histopathological results and immunohistochemistry results were calculated by chi-square test.

OBSERVATION AND RESULTS

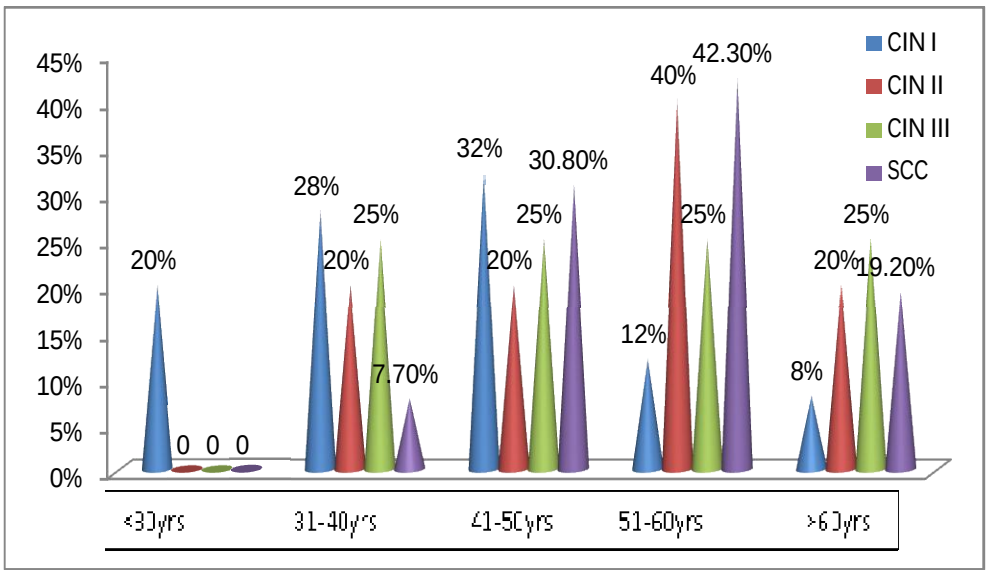
Our present cross sectional study was a prospective study conducted in the Department of Pathology during the period of June 2014 to August 2015. Ethical clearance for the study was obtained from the Ethics Committee of Chengalpattu Medical college, Chengalpattu.

A total sample of 60 cases, including CIN I, CIN II, CIN III, and squamous cell carcinoma of cervix were analysed during the period of June2014 to August 2015.Data obtained were coded and entered into Microsoft excel spread sheet and analysed as below. Results are represented in the form of pie diagrams and bar charts.

TABLE 8: AGE WISE DISTRIBUTION OF CERVICAL SQUAMOUS LESIONS

AGE GROUP	CIN I n = 25	CIN II n = 5	CIN III n = 4	SCC n = 26
< 30 Years	5 (20%)	0	0	0
31-40	7 (28%)	1 (20%)	1 (25%)	2 (7.7%)
41-50	8 (32%)	1 (20%)	1 (25%)	8 (30.8%)
51-60	3 (12%)	2 (40%)	1 (25%)	11 (42.3%)
>60	2 (8%)	1 (20%)	1 (25%)	5 (19.2%)

Figure 13: Age Wise Distribution of Cervical Squamous Lesions



From the above table (8) & figure (13) it is evident that, low grade preneoplastic lesions (CIN I) are seen most commonly in 41 – 50 years of age, high grade lesions (CIN II, III) and squamous cell carcinoma are most commonly seen in 51 – 60 years of age. The age range of the 60 patients in the present study was 27 years to 83 years with a median age 48.5years. Mean age of all patients included in this study was 49.85±12.6. Mean age of CIN I was 42.40years, CIN II was 54.80 years ,CIN III57.25 years and SCC was 54.92 years.

Table 9: Incidence of Preneoplastic and Neoplastic Lesions of cervix in relation to parity

PARITY	CIN I n = 25	CIN II n = 5	CIN III n = 4	SCC n = 26
Nulliparity	2 (8%)	1 (20%)	0	0
1 -2	20 (80%)	2 (40%)	1 (25%)	13 (50%)
3-4	3 (12%)	2 (40%)	3 (75%)	13 (50%)
Chi sq=14.76,P value=0.02				

Figure 14: Incidence of Preneoplastic and Neoplastic Lesions of Cervix in Relation to Parity

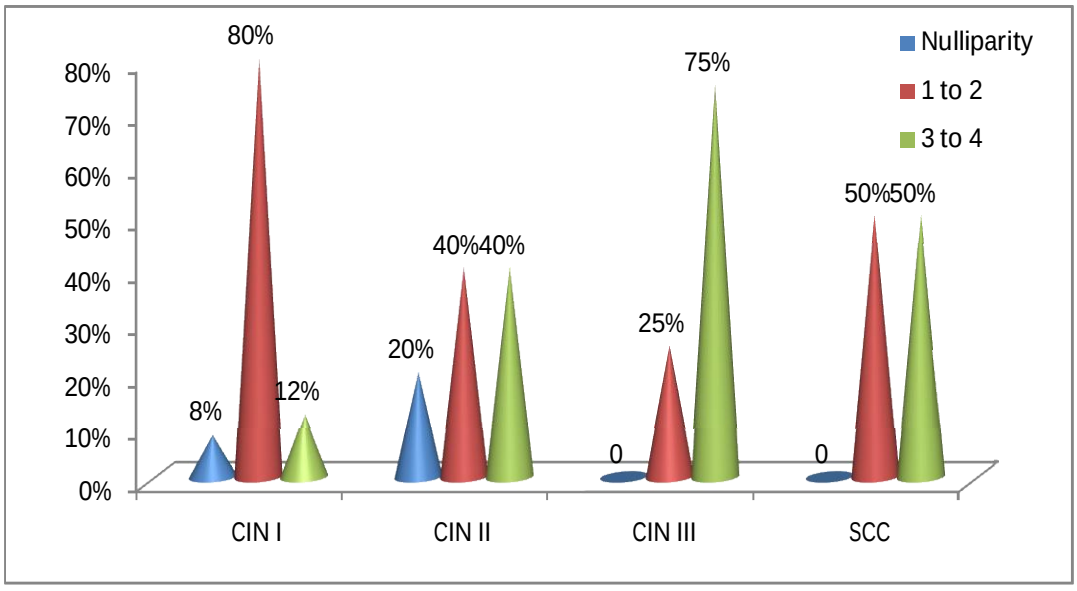


Table and figure mentioned above shows that, in our study low grade squamous intraepithelial lesions (CIN I) were more common in para (1 – 2) women, high grade lesions (CIN II, CIN III) were more common in para (3 –

4) women.In our study, regarding SCC there is no difference in the incidence of SCC in women with para 1- 2 and para 3- 4.

Table 10: Incidence of Various Symptoms in Cervical Squamous Lesions

HPE Diagnosis	SYMPTOMS			
	White Discharge	**Post Coital Bleeding**	**Postmenousal Bleeding**	**Metrorhagia**
CIN (I, II, III)	29 (76.3%)	3 (25%)	0	5 (50%)
SCC	9 (23.7%)	9 (75%)	15 (100%)	5 (50%)
TOTAL	38	12	15	10
Chi sq	18.12	11.03	26.15	3.27
P value	**0.001**	**0.01**	**0.0001**	0.3

In our study it is noted that most of the patients presented with white discharge. The commonest presenting complaint was white discharge in patients with all grades of CIN and post-menopausal bleeding in patients with squamous cell carcinoma of cervix

Figure 15: Incidence of Various Symptoms in Cervical Squamous Lesion.

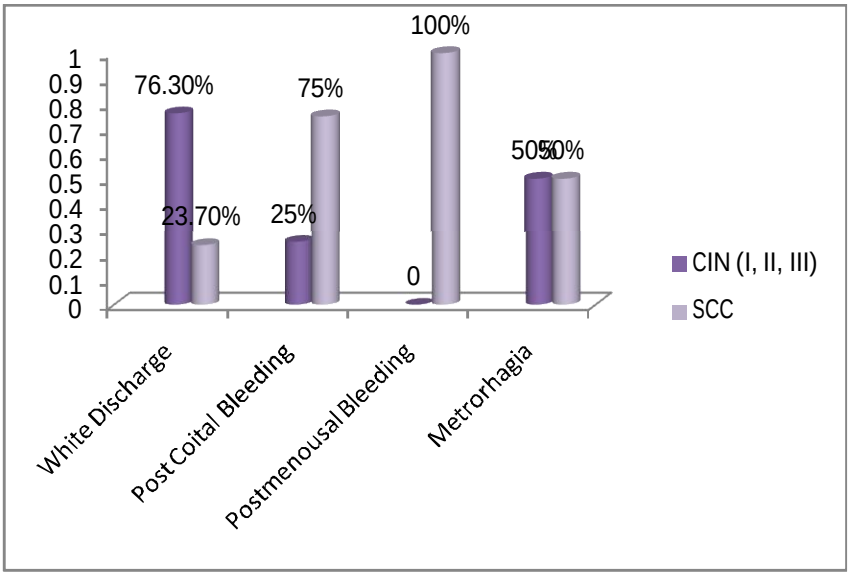


Table 11: Correlation Between Visual Screening Methods and Biopsy Diagnosis

HPE DIAGNOSIS	VIA / VILI POSITIVE
CIN I (n =25)	14 (58.3%)
CIN II (n = 5)	3 (12.5%)
CIN III (n = 4)	1 (4.2%)
SCC (n = 26)	6 (25%)
Total	24

In the present series, out of 60cases VIA / VILI was done for 24 twenty four patients and found positive for all 24 patients. Of the twenty four cases, 14 were diagnosed as CIN I , 3 cases were CIN II ,one was CIN III and 6 cases were diagnosed as squamous cell carcinoma of cervix.

Figure 16: Correlation Between Visual Screening Methods And Biopsy Diagnosis

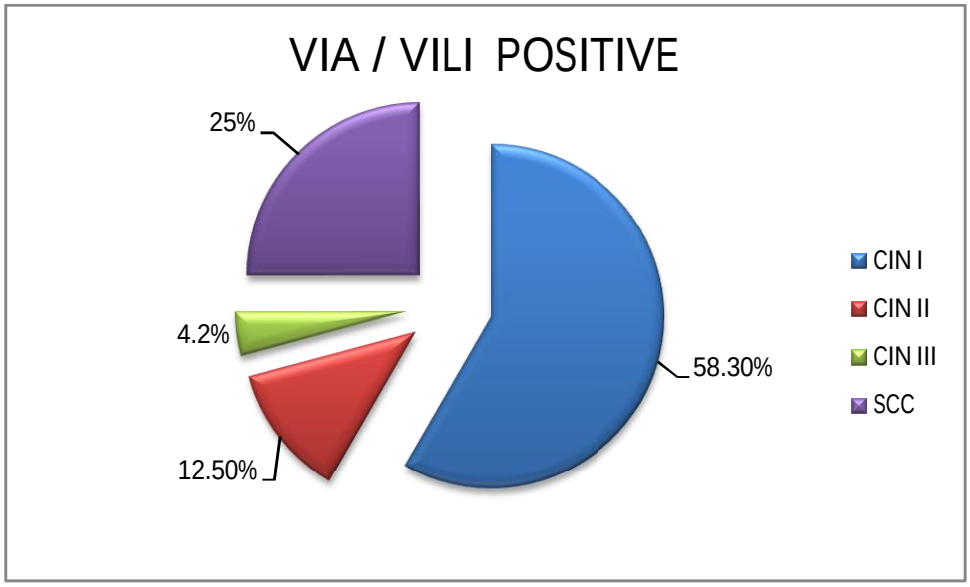
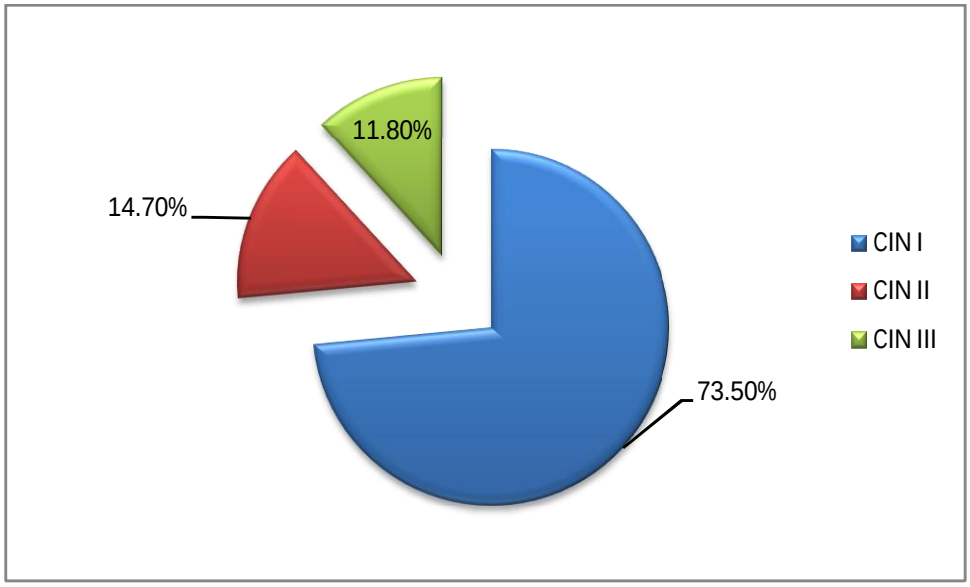


Table 12: Degree of Distribution of Cervical Squamous Intraepithelial Lesion in all Cervical Biopsies.

NATURE OF LESION IN CERVIX	DISTRIBUTION	
	NUMBER OF CASES (n = 34)	PERCENTAGE
CIN I	25	73.5%
CIN II	5	14.7%
CIN III	4	11.8%

Figure17: Degree of Distribution of Cervical Squamous Intraepithelial Lesion in all Cervical biopsies.



In the present study, among 34 cervical intra epithelial lesion , majority of them were CIN I accounting for 73.5%, followed by CIN II with 14.7% and the least being CIN III accounting for 11.8%.

Table 13: Distribution of Various Histological Subtypes of Squamous Cell Carcinoma of Cervix

NATURE OF LESION IN CERVIX	**DISTRIBUTION**	
	NUMBER OF CASES n = 26	**PERCENTAGE**
Early Invasive SCC	2	7.7%
Large Cell Keratinizing SCC	6	23.1%
Large Cell Non-Keratinizing SCC	16	61.5%
Small Cell Non-Keratinizing SCC	2	7.7%

In our study it was noted that, among 26 squamous cell carcinoma cases, 16 cases were diagnosed as Large Cell Non- Keratinizing SCC, 6 cases were diagnosed as Large Cell Keratinizing SCC, 2 cases as Early Invasive SCC and 2 cases as Small Cell Non-Keratinizing SCC.

Figure18: Distribution of Various Histological Subtypes of Squamous Cell Carcinoma of Cervix

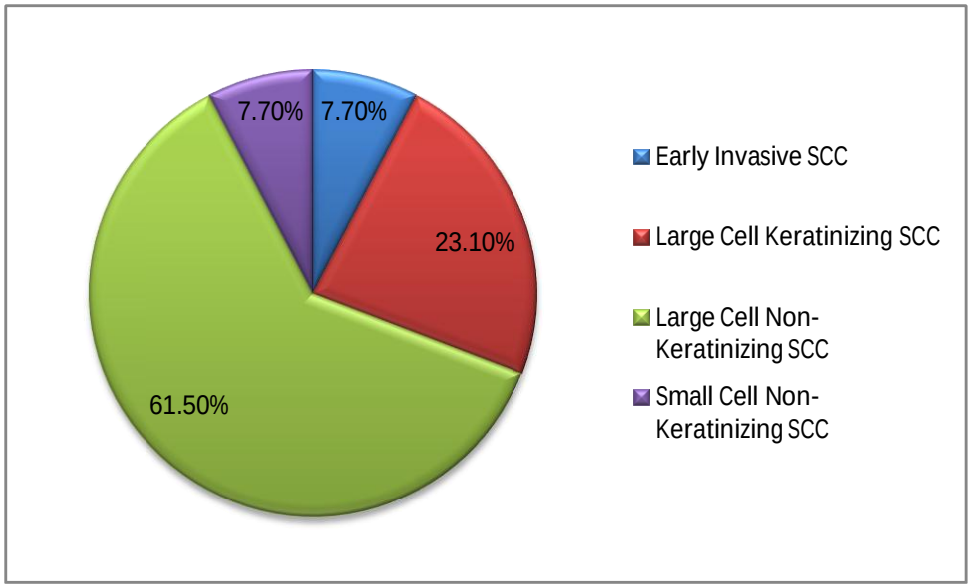


Table 14: Results of P16 Ink4a Immunostaining in Cervical Squamous Cell Lesions

LESION	P16 +ve Cases	p16 negative cases
CIN I	7/25 (28%)	18/25 (72)%
CIN II	4/5 (80%)	1/5 (20%)
CIN III	4/4 (100%)	0
SCC	26/26 (100%)	0
Total	41/60 (68.33%)	19/60 (31.67%)

Chi sq = 33.01, p =0 .0001

Among CIN I group, majority of them (72%) were observed p16 negative in contrast to CIN II , CIN III and SCC, in which most of the cases showed p16 positivity. On making comparison between p16 expression versus different grades of CIN and SCC it was found to be statistically significant (P =0 .0001).

Figure19: Results of P16 Ink4a Immunostaining in Cervical Squamous Cell Lesions

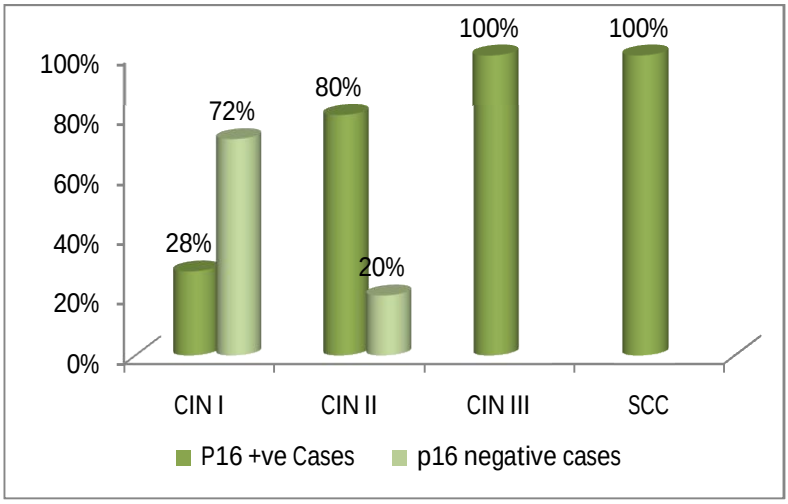


Table 15: Grading of P16 Ink4a Expression in Preneoplastic and Neoplastic Squamous Cell Lesions of Cervix

LESION	-Ve	Grade 1	Grade 2	Grade 3
CIN I	18/25 (72)%	3/25 (12%)	3/25 (12%)	1/25 (4%)
CIN II	1/5 (20%)	0	1/5 (20%)	3/5 (60%)
CIN III	0	0	0	4/4 (100%)
SCC	0	0	1/26 (3.85%)	25/26 (96.15%)

Chi sq = 50.26, p =0 .0001

Above mentioned table showed that expression of $p^{16INK4a}$is increased with increasing grades of CIN and also in squamous cell carcinoma. Majority of the CIN I cases(72%) werep16 negative.p16 expression in CIN II was 80% and CIN III was 100%. 4% of CIN I case, 60% of the CIN II cases and 100% of the CIN III cases showed grade 3 staining. Most of the SCC (96.15%) showed grade 3 scoring for p16 positivity except one case which showed grade 2 scoring.

Figure 20 : Grading of P16Ink4a Expression in Preneoplastic and Neoplastic Squamous Cell Lesions of Cervix

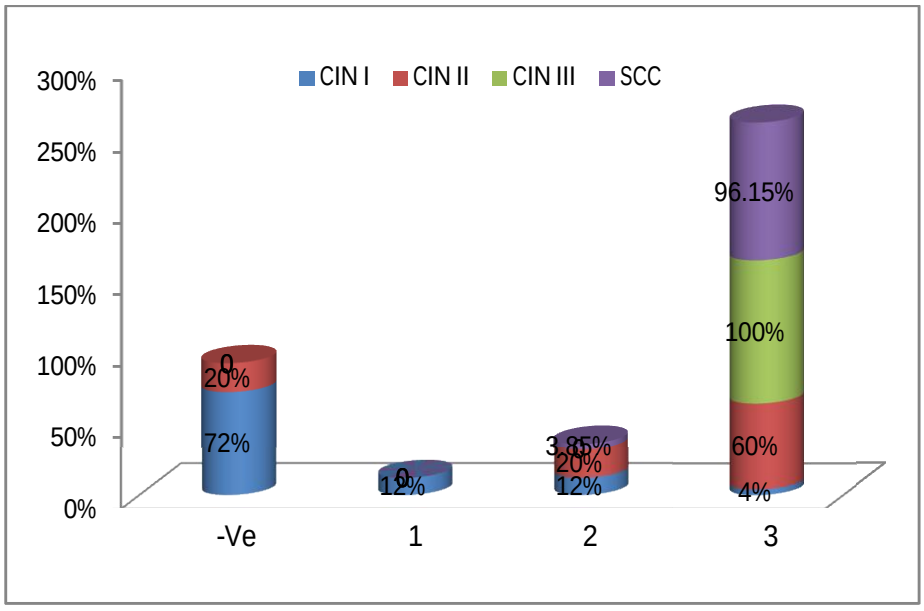


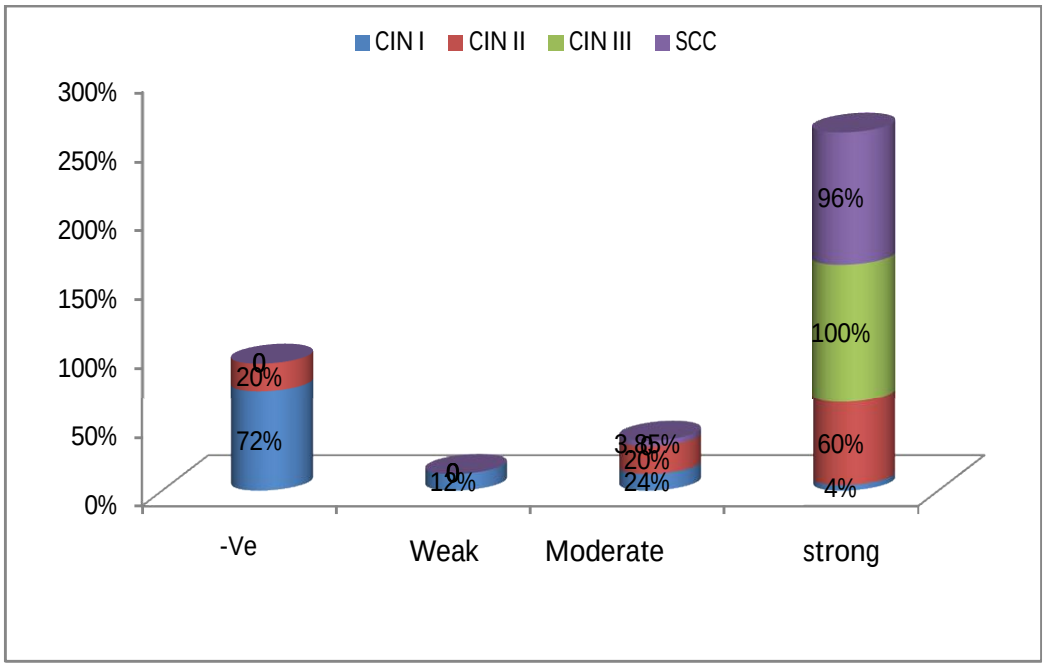
Table 16: Correlation Between Histopathological Diagnosis and Reaction Intensity of P16 Staining:

LESION	-Ve	Weak	Moderate	Strong
CIN I	18/25 (72%)	0	6/25 (24%)	1/25 (4%)
CIN II	1/5 (20%)	0	1/5 (20%)	3/5 (60%)
CIN III	0	0	0	4/4 (100%)
SCC	0	1/26(3.85%)	1/26 (3.85%)	24/26 (96%)

Chi sq = 52.49, p = 0.0001

In the present study it was noted that except CIN I , majority of the CIN II, III and SCC showed strong reaction intensity for p16 immunostaining.

Figure 21: Correlation Between Histopathological Diagnosis and Reaction Intensity of P16 Staining:



COLOUR PLATES

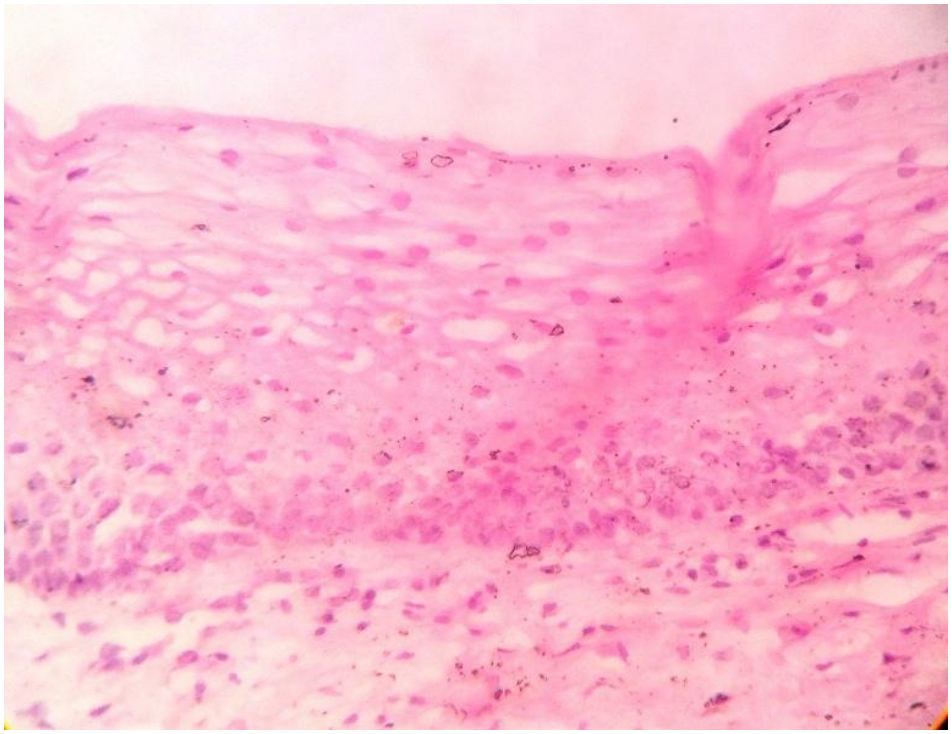


Figure 22 : Cervical intraepithelial neoplasia I (CIN I) . H&E (100X)

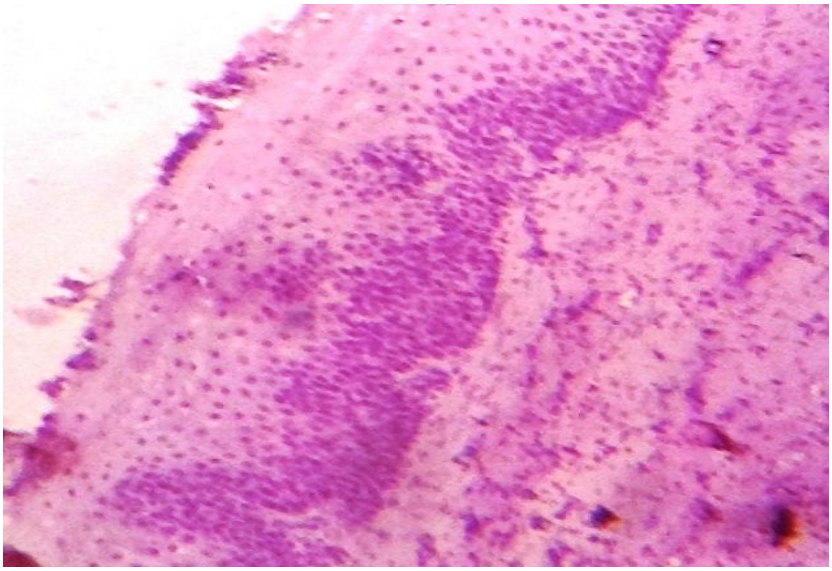


Figure23 : Cervical intraepithelial neoplasia I (CIN I) . Negative p16 IHC staining (100X)

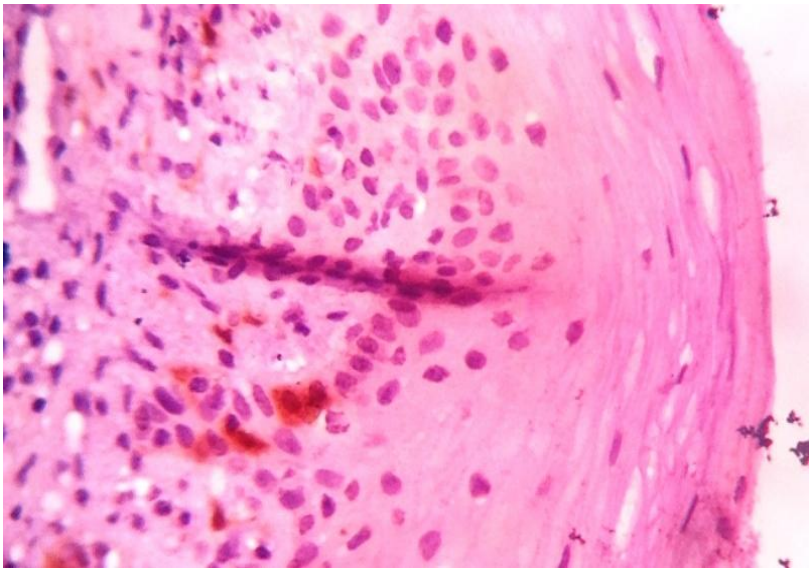


Figure24 :Cervical intraepithelial neoplasia I (CIN I) . p16 IHC staining Grade 1 staining. (400X)

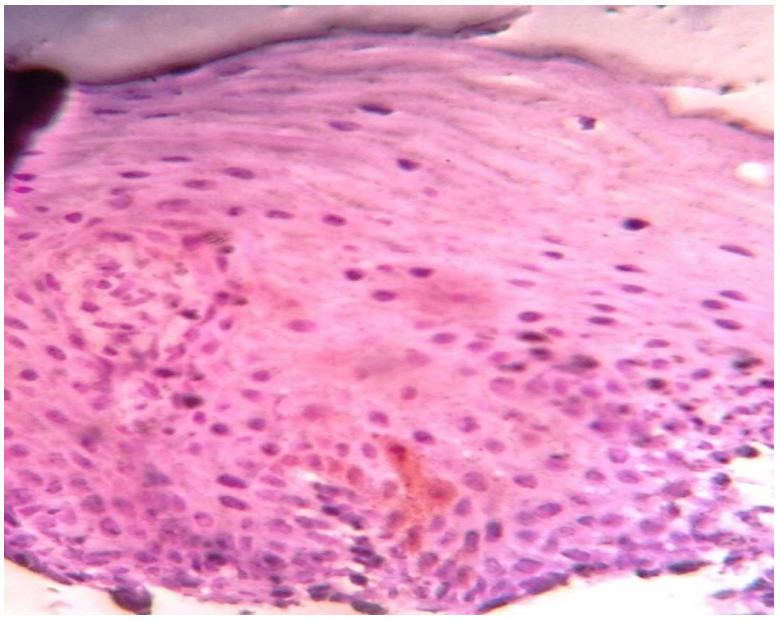


Figure25 :Cervical intraepithelial neoplasia I (CIN I) . p16 IHC staining Grade 2 staining. (400X)

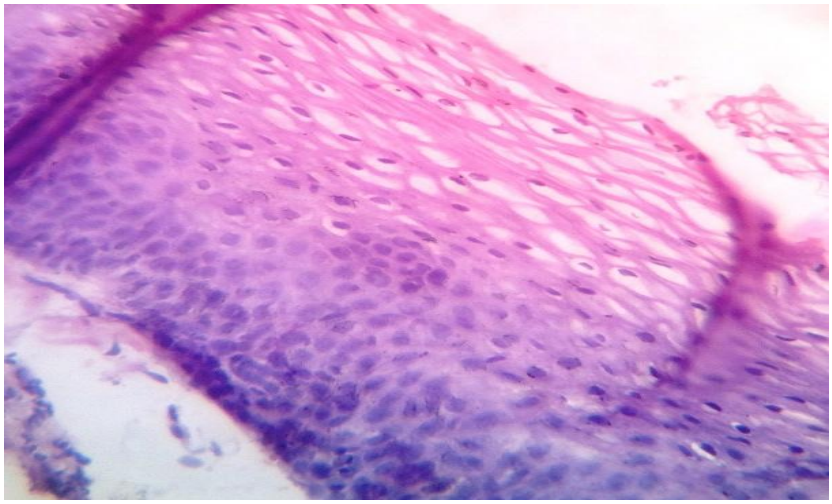


Figure26 :Cervical intraepithelial neoplasia II (CIN II) . H&E (100X)

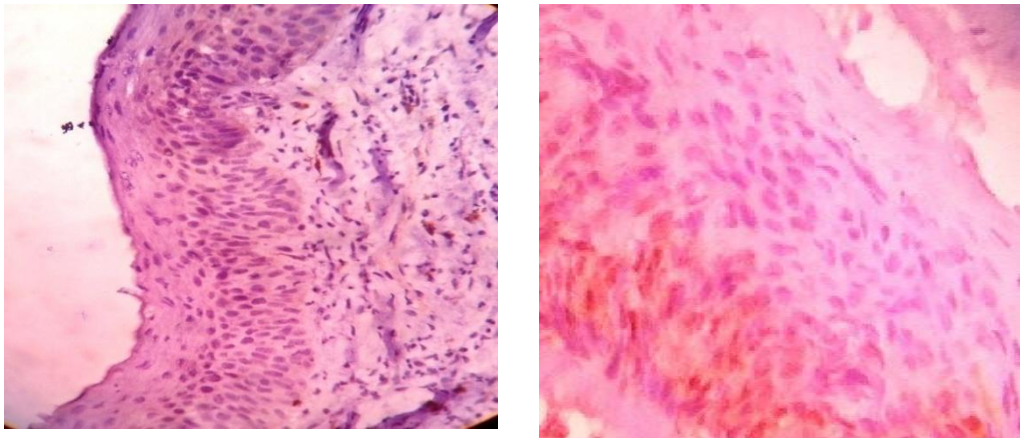


Figure27 : Cervical intraepithelial neoplasia II (CIN II) . p16 IHC staining Grade 2,3 staining. (400X)

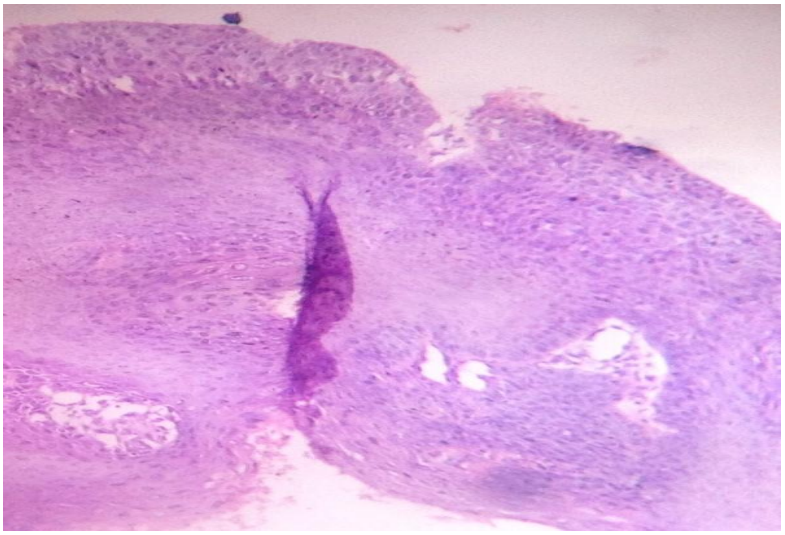


Figure28 : Cervical intraepithelial neoplasia III (CIN III) . H&E (100X)

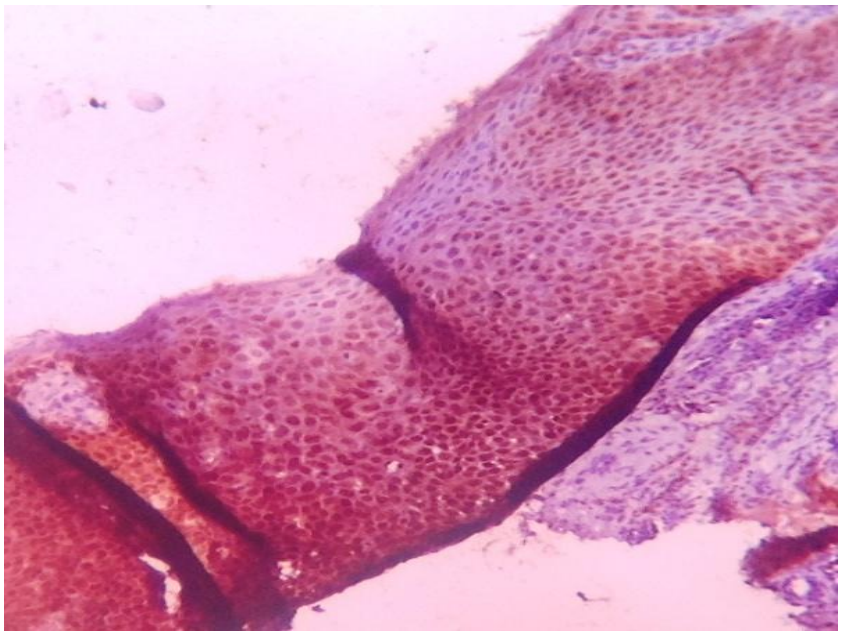


Figure 29: Cervical intraepithelial lesion III (CIN III).P16 IHC immunostaining Grade 3 (100X)

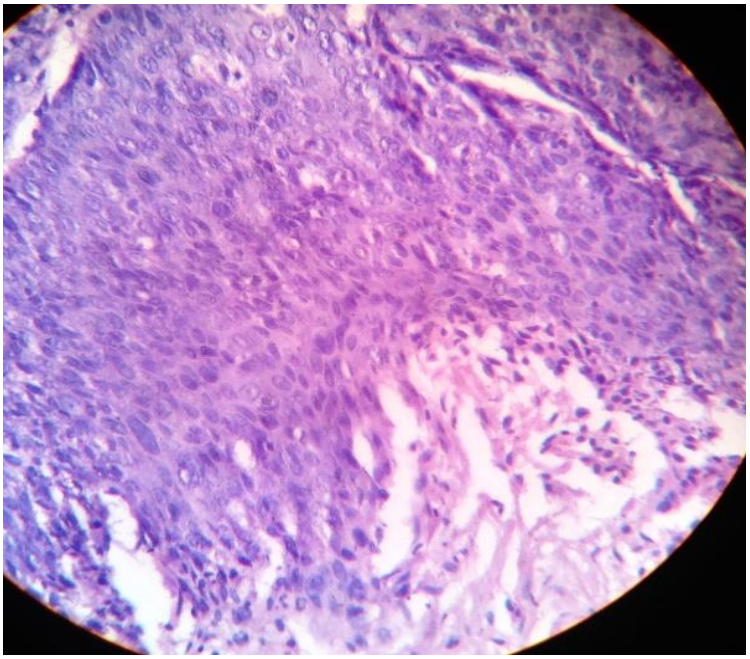


Figure30 : Early invasive squamous cell carcinoma. H& E.(100X)

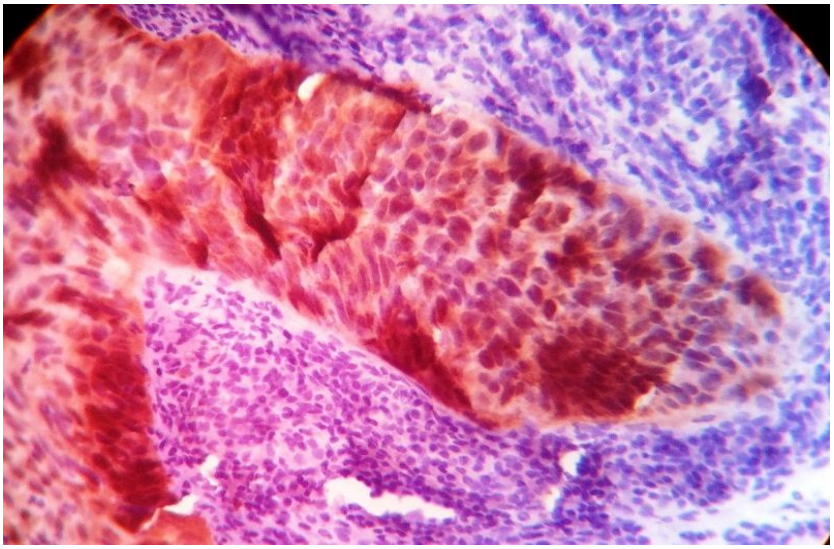


Figure 31: Early invasive squamous cell carcinoma.P16 immunostaining shows grade 3 immunostaining (400X)

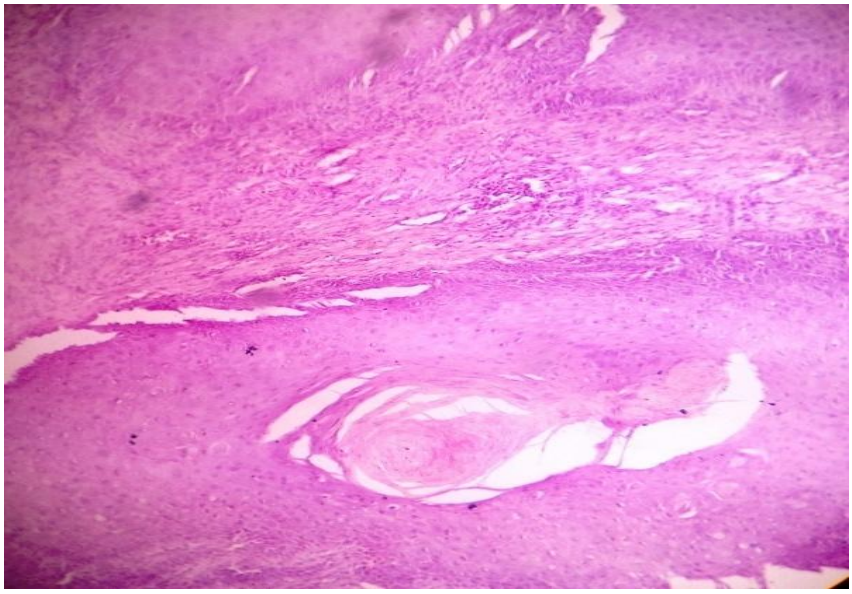


Figure 32 : Large cell keratinizing squamous cell carcinoma. H& E. (100X)

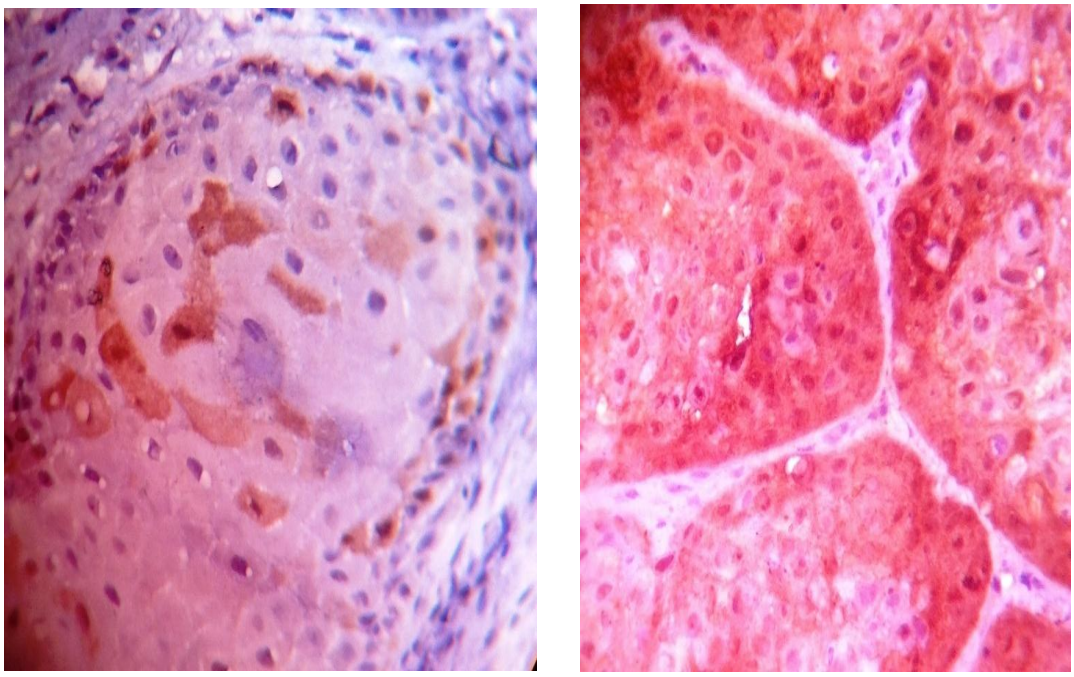


Figure 33: Large cell keratinizing squamous cell carcinoma.P16 immunostaining shows grade2& 3 immunostaining(400X)

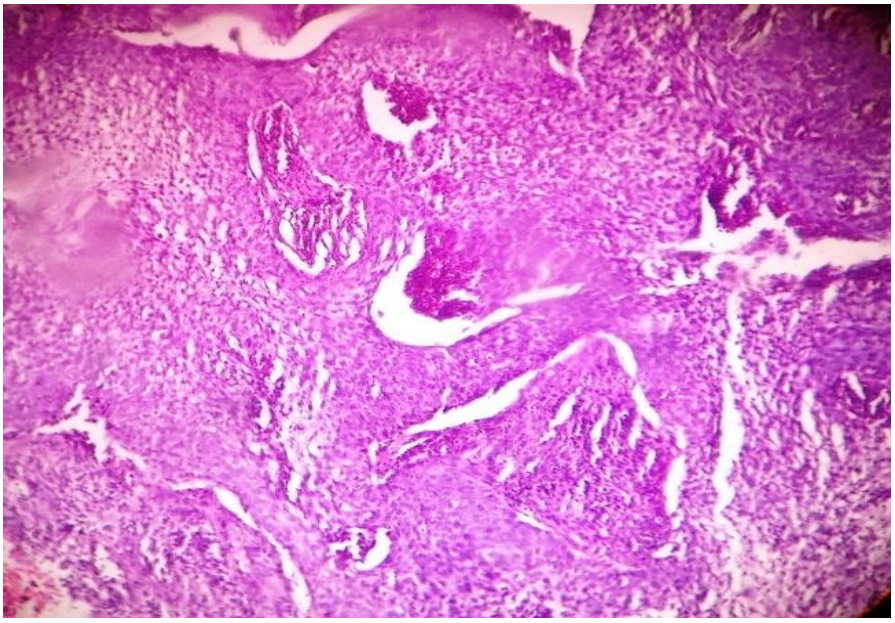


Figure 34: Large cell non keratinizing squamous cell carcinoma. H& E.(100X)

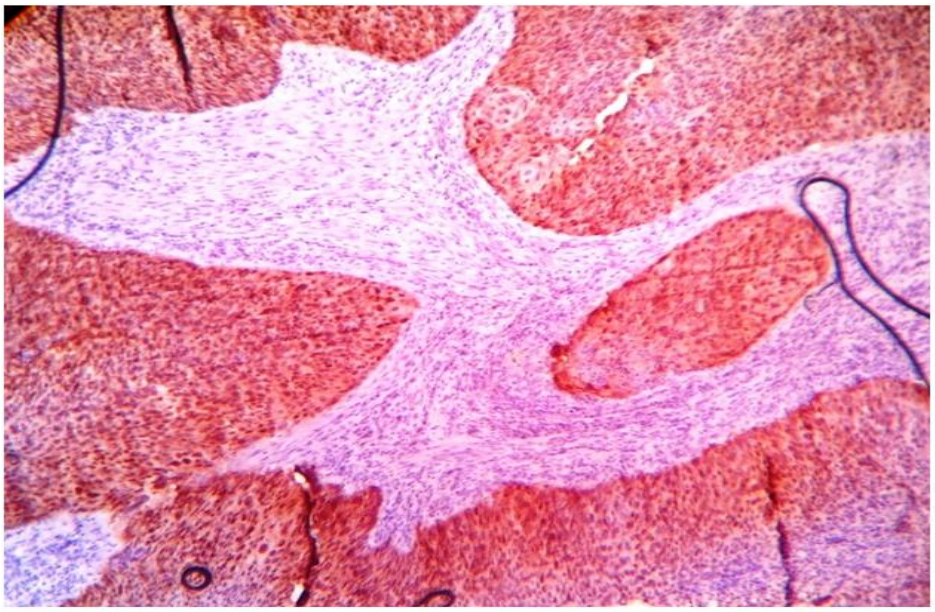


Figure 35: Large cell non keratinizing squamous cell carcinoma.P16 immunostaining shows grade 3 immunostaining

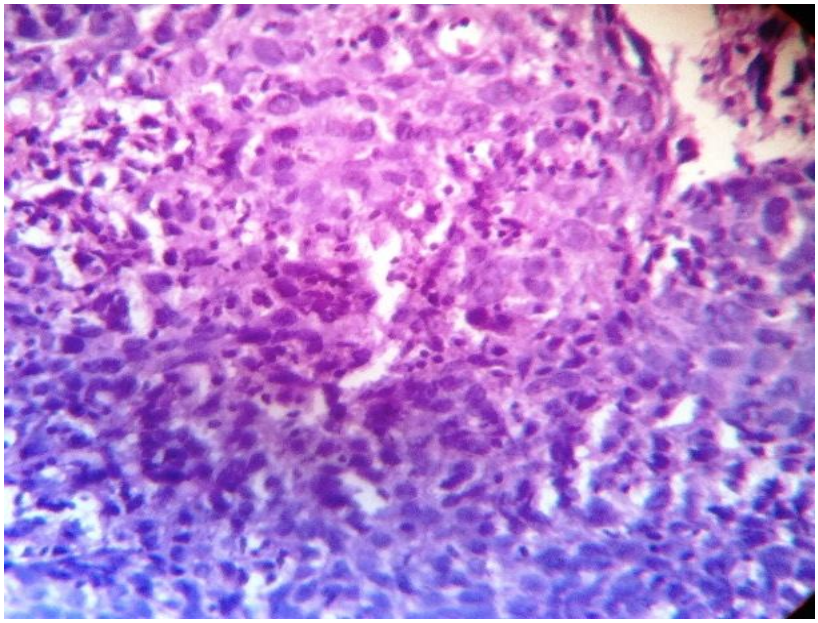


Figure 36: Small cell non keratinizing squamous cell carcinoma. H& E.(100X)

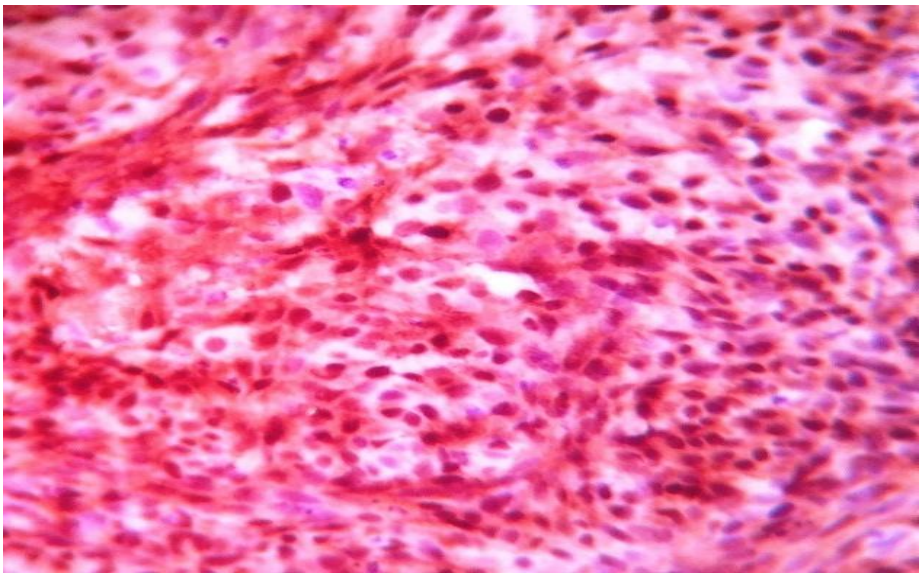


Figure37: Small cell non keratinizing squamous cell carcinoma.P16 immunostaining shows grade 3 immunostaining

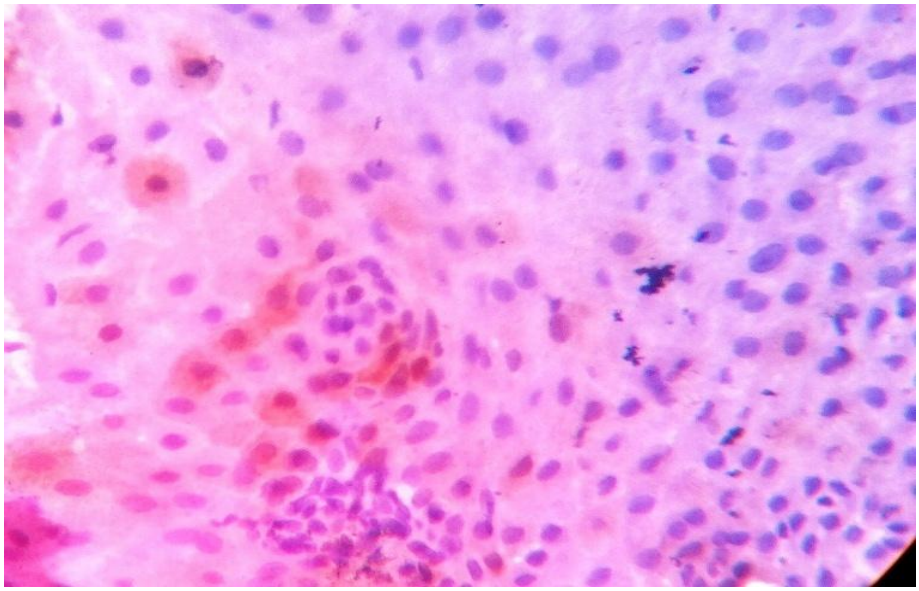


Figure 38: p16 immunostaining showing weak reaction intensity

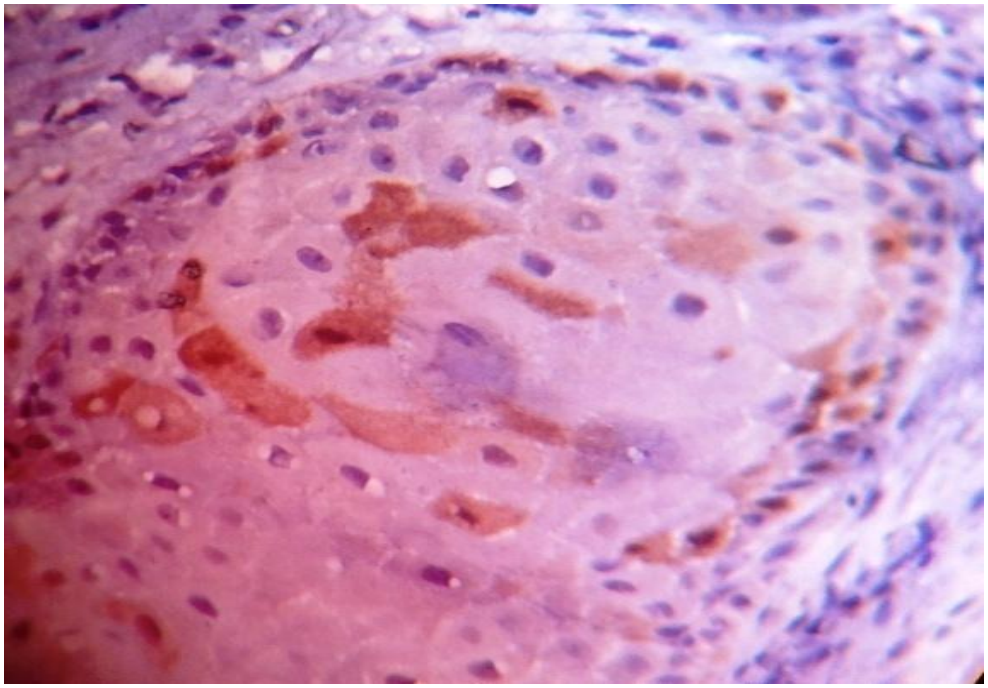


Figure 39: p16 immunostaining showing moderate reaction intensity of p16 staining

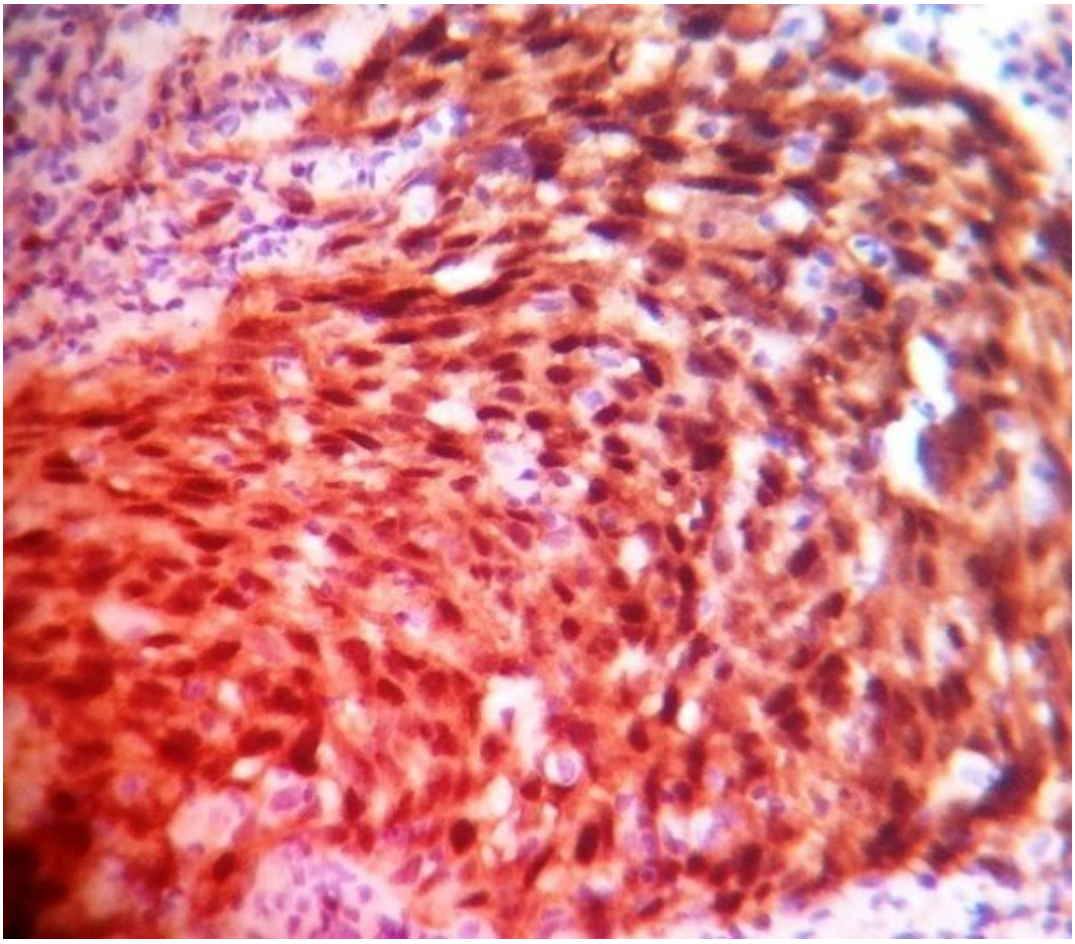


Figure 40: p16 immunostaining showing strong reaction intensity of p16 staining.

DISCUSSION

Cervical cancer is one of the leading causes of morbidity and mortality among women worldwide. Many studies revealed the association of human papilloma virus infection in both precancerous and invasive cervical cancer.[97] Most of the HPV infection are transient, if it persists the risk of developing preneoplastic lesions increases as well as the risk of developing cervical cancer.[98]

Among various types of cervical carcinoma, squamous cell carcinoma constitutes majority which is preceded by cervical intraepithelial lesion (CIN) and the later plays an important critical point in the natural history of cervical cancer.

Pap smear screening and histopathological examination and interpretation of cervical biopsy specimen has markedly reduced the number of deaths due to cervical cancer, however they give little information regarding the association of HPV infection, risk of progression or regression and prognosis. Most of the low grade intraepithelial lesion and some of the high grade lesion regress spontaneously overtime without treatment.[99] So overtreatment of patients who will not benefit from treatment or under treatment of patients who have the risk of progression, underscores the importance of detection of HPV in those lesions. Furthermore absence of detection of HPV in cervical cancer cells is associated with poor prognosis.[100] Hence HPV detection in those lesions play a pivitol role to differentiate HPV

positive from HPV negative SCC by which we can assess the prognosis of the patient.

P16 has recently emerged as a surrogate marker of HPV, used both in cytology and histology sections, it significantly reduce the interobserver variability when diagnosing cervical intraepithelial lesion, furthermore it's over expression appears to correlate with degree of cervical intraepithelial neoplasia.[101]

P16 over expression in low grade cervical intraepithelial lesion have a higher risk of persistence and progression to high grade lesion.[102]In future we can plan HPV vaccine trials in areas with HPV high prevalence. So identifying the association of HPV in preneoplastic and neoplastic lesions has significant implication in diagnostic, follow-up , prognostic and preventive aspects of cervical cancer.

In the present study total number of cervical biopsy sampled were 60.The overall age range of the 60 patients was 27 – 83 years with mean of 49.85years and median age of 48.5 years. The observations by Kim et al and our study are coinciding.

TABLE 17: AGE COMPARISON

AGE PARAMETERS	Kim et al 2015[103]	Benevolo et al 2006[104]	Poste et al 2015[105]	Present study
Age range	24 –80 yrs	18 -82 yrs	29 – 82 yrs	27 -83 yrs
Median age	47 yrs	41 yrs	-	48.5 yrs
Mean age	-	41 yrs	-	49.85 yrs
Common age range of CIN	-	-	41 – 50 yrs	41 – 50 yrs
Common age range of SCC	-	-	51- 60 yrs	51- 60 yrs

Majority of low grade lesions were seen in 41 – 50 years and SCC were seen in 51 – 60 years age group ,which is similar to study done by poste et al. In our study patients with mean age of high grade CIN(mean 56.02),and SCC (mean 54.92) were older compared to CIN I (mean 42.40).

Table18: Comparison of Incidence of Parity Among Preneoplastic and Neoplastic Lesions of Cervix

Parity	Urmila Banik et al[106] (2011) n = 139	Present study n = 60
0 - 2	24 (17.27%)	39 (65%)
3 - 4	52 (37.41%)	21 (35%)
>5	63 (43.32%)	--

Majority of the patients in the present study were primi para and second para. Many studies have shown that multiparity is one of the risk factor associated with development of carcinoma. In Urmila et al study majority of the patient was parity of > 5. In the present study, no significant correlation exists between multiparity and SCC.

Table 19: Comparison of incidence of various symptoms in CIN & SCC

Symptoms	Kamna Gupta etal 2013		Present Study		Poste etal (CIN) 2015 (n=51)
	CIN (n=87)	SCC (n=11)	CIN(n=34)	SCC(n=26)	
White discharge	42 cases (48.27 %)	0%	29 cases (85.29%)	9 cases (34.62%)	17 cases 33.33%
Post coital bleeding	23 cases (26.43%)	4 cases (36.36%)	3 cases (8.82%)	9 cases (34.62%)	10 cases 19.6%
Post menopausal bleeding	6 cases (6.90%)	5 cases (45.45%)	-	15 cases (57.69%)	1 case 1.96%
Metrorrhagia	15 cases (17.24%)	2 cases (18.18%)	5 cases (14.7%)	10 cases (38.46%)	19 cases 37.25%

From the above table it is seen that most common complaint in patients with CIN was white discharge and in patients with SCC was post menopausal bleeding. Our finding is concordant with Kamna gupta et al.[107] But in poste et al the predominant complaint of CIN patients was metrorrhagia.

In our study VIA / VILI was done for 24 cases and it was found positive in all 24 cases (100%). Out of 24 cases, 14 cases(58.3%) were diagnosed as CIN I, 3 cases(12.5%) were diagnosed as CIN II(12.5%) , one case(4.2%) as CIN III and 6 cases(25%) were diagnosed as SCC. A study by Ghosh et al in 2010 observed that among 34 VIA / VILI positive cases, 11cases (32.35%) had CIN I, 4 cases (11.4%) had CIN II, 1case (2.94%) had CIN III and 1case (2.94%) had invasive cancer. In low resource countries pap smear screening can be replaced or combined with VIA /VILI to detect precancerous and cancerous lesions.

Table 20: Comparison of Incidence of CIN with other Studies

CIN GRADE	**Sophia S Wang et al[108] (2014) (no of cases) (n = 292)**	**Tan et al[109] (2010) (no of cases) (n = 129)**	**Nam et al[110] (2008) (n = 24)**	**Present study (n = 60)**
CIN I	75 cases	60 cases	12cases	25 cases
CIN II	19 cases	21 cases	6cases	5 cases
CIN III	19 cases	48 cases	6cases	4 cases

In the present study of 60 cases , CIN I constituted the major group accounting for about 73.5% followed by CIN II, III , accounting 14.7% , 11.8% respectively. The lesion with highest incidence was CIN I in our study and is similar to the incidence quoted by Wang et al, Tan et al and Nam et al.

Table 21: Comparison of Distribution of Various Histological Subtypes of Squamous Cell Carcinoma

SUPTYPE OF SCC	Purnima poste et al (2015) (n = 157)	Vatsala misra et al (1997)[111] (n = 98)	Adisorn Jedpiyawongse et al (2008)[112] (n = 29)	Present study No of cases (%) (n = 26)
Early invasive SCC	-	-	13(25%)	2(7.7%)
Large cell keratinizing SCC	48(30.5%)	18(18.37%)	1(1.8%)	6(23.1%)
Large cell keratinizing SCC	103(65.60%)	68(69.39%)	14(26.4%)	16(61.5%)
Small cellnon keratinizing SCC	6(3.82%)	4(4.08%)	1(1.8%)	2(7.7%)

Among SCC, majority of them were of large cell non keratinizing subtype. The incidence of this is similar to the incidence quoted by other authors. Non keratinizing tumors of cervix had better prognosis when radiotherapy is used, but there was no significant difference in the prognosis when the treatment is surgical.

Table 22: Comparison of P16 Positivity in CIN & SCC with Other Studies

Sl. no.	Authors	CIN1	CIN2	CIN3	SCC
1.	Klaes et al 2002	15/17 (88%)	10/10 (100%)	43/43 (100%)	46/46 (100%)
2.	Benovolo 2006	17/54 (31%)	9/10 (90%)	11/11 (100%)	8/8 (100%)
3.	Kim et al 2015	22/31 (70.9%)	21/25 (84%)	41/41 (100%)	35/35 (100%)
4.	Tan et al 2010	16/60 (26.7%)	9/21 (42.9%)	46/48 (95.9%)	71/72 (98.6%)
5.	Present Study	7/25 (28%)	4/5 (80%)	4/4 (100%)	26/26 (100%)

In SCC, 100% p16 positivity was noted in our and other studies. In CIN III p16 positivity (100%) was correlate with other studies. In CIN II p16positivity (80%) was correlated with other studies except Tan et al. In CIN I , the positivity(28%) correlated with Benovolo et al and Tan et al. Among preneoplastic lesions, totally 44.12% of cases including CIN I, II, III showed p16 positivity.

In the present study, we observed p16 negativity in majority of the CIN I samples (72%). Sano T et al indicated that low risk HPV E7 oncoprotein have no effect on p16 expression, because its affinity is 10 times lower than that high risk HPV E7 oncoprotein.[113] Tan et al in 2010 stated that low grade lesion with p16 negativity may be due to infection with low risk HPV or due to subclinical infection.

In the present study all the CIN III and SCC cases were p16 positive. The results of expression of p16 in all grades of CIN and SCC was statistically significant (p =0.0001) and is concordant with the above literatures.

A study by volgareva et al in 2004, observed that some of the preneoplastic and neoplastic lesions of cervix do not express p16. They suggested that due to lack of p16 positivity we should not exclude a patient from risk group. They concluded that absence of p16 expression may be due to p16 mutation, deletion or hypermethylation.[114]

Table 23 : Comparing Grade of P16 Expression in Squamous Cell Lesions of Cervix.

Study	**P16 Expression grading**								
	Lesion	**Negative**		**Grade 1**		**Grade 2**		**Grade 3**	
		N	%	n	%	n	%	n	%
Kleas et al (2001)[115]	CIN I n=47	7	14%	2	4%	10	21%	29	61%
	CINII n=32	-	0%	-	0%	-	0%	32	100%
	CINIII n=60	-	0%	-	0%	-	0%	60	100%
	SCC n=60	2	3%	-	0%	-	0%	58	97%
Supriya Srivastava et al (2010)[116]	CINI n=10	2	20%	-	0%	8	80%	-	0%
	CINII n=5	-	0%	-	0%	3	60%	2	40%
	CINIII n=3	-	0%	-	0%	-	0%	3	100%
	SCC n=15	-	0%	-	0%	-	0%	15	100%
Present study	CINI n=25	18	72%	3	12%	3	12%	1	4%
	CINII n=5	1	20%	-	0%	1	20%	3	60%
	CINIII n=4	-	0%	-	0%	-	0%	4	100%
	SCC n=26	-	0%	-	0%	1	3.85%	25	96.15%

In the present study it was noted that there was a significant increase in the grade of p16 expression, when we moved from low grade cervical intraepithelial lesion to squamous cell carcinoma and the correlation was found to be statistically significant (p value = 0.0001%).In SCC, 96.15% of cases showed grade 3 expression.

Kleas et al in 2001 correlated the p16 expression in cervical lesions to the HPV status of the sample by using PCR. They grade the p16 expression as negative , sporadic ,focal and diffuse. All CIN I lesions with low risk HPV showed no diffuse staining, but CIN I with high risk HPV displayed diffuse p16 expression . Finally they concluded that p16 may be useful to identify the dysplastic cervical cells in both cytology and histopathology. In the present series one CIN I case showed grade 3 expression of p16 which need greater attention, because of increased risk of persistence and progression to high grade lesion. So p16 could be used asa suitable marker of HPV infection to predict the outcome of CIN lesions.

Ishikawa et al analysed the p16 expression and HPV typing in cervical lesions and found that p16 overexpression in CIN I was more common in patients with HPV 16 and 52.[117]

A study by supriya srivastava(2010) et al analyzed the expression of both p16 and MIB1 in cervical lesions and normal cervical epithelium. They found the expression of both in all cases of CIN I, II, III and cancer cervix except in normal cervical epithelium. In their study they grade the number of p16 positive cells as grade 0(0% positive cells) ,1(1 – 10%positive cells) ,2(10 – 50% positive cells) and 3 (>50%p16 positive cells).

A study by Riou G et al (1990) examined 106 cases of early invasive squamous cell carcinoma of cervix with HPV sequencing by PCR and southern hybridization and concluded that there was 2.6 times higher chance of relapse and 4.5 times higher chance of distant metastasis in HPV negative patients when compared to HPV positive patients. So detection of HPV in those lesions indicate better prognosis.

Table 24: Correlation between p16 Reaction Intensity and Histological Diagnosis with Other Studies

Study	HPE Diagnosis	P16 reaction intensity			
		Negative	Weak	Moderate	Strong
Izadi Mood et al 2012	Low grade CIN (n = 11)	2 (18.2 %)	-	9 (81.8%)	-
	High grade CIN(n = 11)	1 (9.09 %)	-	2 (18%)	8 (72.7%)
	SCC(n = 20)	2 (10%)	1 (5%)	2 (10%)	15 (75%)
Present Study	Low grade CIN (n = 25)	18 (72%)	-	6(24%)	1(4%)
	High gradeCIN (n = 9)	1 (11.11%)	-	1(11.11%)	7(77.77 %)
	SCC (n =26)	-	1(3.85%)	1(3.85%)	24(96.15%)

Izadi mood et al in 2012 observed a direct relationship between reaction intensity and lesion severity. They concluded that p16 reaction

intensity was superior than any other analyzed parameter and they found it being the best indicator of p16 expression.[119]

In the present study majority of the low grade CIN were negative for p16 reaction intensity, but all other findings were concordant with the above literature. There was a statistically significant relationship between histopathological diagnosis and p16 reaction intensity in our study ($p < 0.0001$).

SUMMARY

Cervical cancer is one of the most common malignancy among women worldwide.HPV plays an important role in the development of premalignant and malignant tumors of cervix. Routine screening methods may not detect HPV in those lesions. Hence the present study was undertaken to know the association of HPV in those lesions, which is very useful to predict the progression of the disease. In our study all the 60 cases were analyzed with p16 INK4A marker , which is a surrogate marker of HPV.

The overall age range of patients in this study was between 27 years to 83 years, with a mean of 49.85 years and median of 48.5 years. Majority of the patients with low grade CIN belonged to the age group of 41 – 50 years, and SCC belonged to 51 – 60 years.

Most of the patients in this case series were para 1-2.High grade lesions were seen in para 3-4 patients.

White discharge and Metrorrhagia were the most common symptoms of patients with CIN, it was seen in 76.3% of patients, whereas post menopausal bleeding was the most common symptom of squamous cell carcinoma patients.VIA/ VILI was found positive in 100% of the tested patients

In this study OUT OF 60 cases CIN constituted 56% and SCC constituted 44%.Among all CIN cases, CIN I constituted majority of the cases. Large cell non keratinizing SCC was the commonest subtype of SCC comprising 61.5%.

Overall in 60 cases, 68.33% of patients sample showed p16 expression, out of this 28% of CIN I, 80% of CIN II and all CIN III and all SCC cases showed p16 expression. One CIN I case and majority of the CIN 2, 3 and SCC cases scored grade 3 p16 staining.

In the current study 4% of the CIN I, 60% of CIN II, 100% of CIN III and 96.15% of SCC cases showed strong reaction intensity for p16 staining.

In our study we found statistically significant relationship between p16 expression, reaction intensity and histopathological diagnosis.

CONCLUSION

The present cross sectional study titled "Analysis of Immunohistochemical Expression of p16INK4a in preneoplastic and neoplastic squamous cell lesions of cervix" was conducted in the department of pathology from June 2014 to August 2015.

Following conclusions were arrived from this study:-

- Low grade lesions were seen in younger age group(41 – 50 years) compared to high grade lesions which were seen in older age group (51 – 60years).

- Among preneoplastic lesions, low grade cervical intraepithelial lesions were found in low parity women and high grade lesions were seen in high parity women. In invasive squamous cell carcinoma, there is no significant relation with high parity.

- VIA / VILI showed 100% positivity for all the tested cases.

- As nearly 70% of all preneoplastic and neoplastic lesions of cervix showed p16 over expression in our region, further studies can be undertaken to evaluate the prevalence of high risk HPV in general population, which can help us to take necessary steps to minimize and prevent the infection through health education and HPV vaccination.

- Low grade cervical intraepithelial lesions showed less reaction intensity and less grade of staining. P16 expression was progressively increased with increasing grades of cervical neoplasm. So p16 may be useful as

an adjunct in histological sections to detect HPV in those lesions which can help us to predict the progression of disease. High grade cervical intraepithelial lesions and squamous cell carcinoma cases showed strong reaction intensity and higher grade of staining. If we detect the association of HPV in SCC, we can predict the prognosis of the patient.

- The limitation of our study was we did not attempt for HPV DNA detection studies to validate the utility of p16 for detection of HPV in cervical neoplasm.

.

BIBLIOGRAPHY

1. WHO/ICO Information centre on HPV and cervical cancer (HPV information centre).Human papilloma virus and related cancers in India. www.who.int/hpvcentre.

2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray,GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet].Lyon, France: International Agency for Research on Cancer; 2013.

3. M. Evander, K. Edlund, A. Gustafsson et al., "Human papillomavirus infection is transient in young women: a population-based cohort study," Journal of Infectious Diseases, vol. 171, no. 4, pp. 1026–10s, 1995.

4. Fenoglio CM,Galloway DA,Crum CP etal.Herpes simplex virus and cervical neoplasia.In: Fenoglio CM,Wolff M,eds. Progress in surgical pathology.New York: Raven press,1982,pp.45-82.

5. Brinton LA, Reeves WC , Brenes MM et al.The male sexual factor in the etiology of cervical cancer among sexually monogamous women.Int J Cancer1989;44:199.

6. Butter worth CE,Hatch KD,Macaluso M.Folate deficiencyand cervical dysplasia.J Am Med Assoc 1992;267:528.

7. Zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2: 342-50.

8. IARC Monographs on the evaluation of carcinogenic risks to human.Lyon France: 2007:Volume 90; Page no;47.

9. P. Basu, S. Roychowdhury, U. D. Bafna et al., "Human papillomavirus genotype distribution in cervical cancer in India: results from a multi-center study," Asian Pacific Journal of Cancer Prevention, vol. 10, no. 1, pp. 27–34, 2009.

10. Iana Leniskova,Marianne Lidang,Stephen Hamilton-Dutoit, Jørn Koch.p16 as a diagnostic marker of cervical neoplasia: a tissue microarray study of 769 archival specimens.Diagnostic pathology 2009,4:22 doi:10.1186/1746-1596-4-22.

11. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsagué X,; HPV PATRICIA Study Group (2012) Overallefficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervicalintraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind patricia trial. Lancet Oncol 13: 89-99.

12. Wheeler CM, Castellsague X, Garland SM, Szarewski A, Paavonen J, Naud P, (2012) Crossprotectiveefficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infectionand precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-studyanalysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 13: 100-10.

13. U. Saxena, C. Sauvaget, and R. Sankaranarayanan, “Evidence based Screening, early diagnosis and treatment strategy of cervical cancer for national policy in low resource countries: example of India,”Asian Pacific Journal of Cancer Prevention, vol. 13, pp. 1699–1703, 2012.

14. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–2917

15. J. Ferlay, F. Bray, P. Pisani, and D. M. Parkin, GLOBOCAN, 2002 Cancer Incidence, Mortality and Prevalence Worldwide, Version 1. 0. IARC Cancer Base No. 5, IARC Press, Lyon, France, 2004.

16. Inderbir Singh. Textbook of Human Embryology. 9rd Ed. Macmillan Publishers, 2013; 248 – 263.

17. Susan S. Gray’s Anatomy, 40th ed. UK-Churchill Livingstone, Elsevier 2008.

18. Ferenczy A. Anatomy and histology of uterine corpus 1994; In Blaustein’s Pathology of Female genital tract. Kurman R.J. (edi) 4th edition, New York, springer – verleg.

19. Kleeman SD, Silva WA: Gynecologic anatomy. In: Sokol AI, Sokol ER, eds. The Requisites in Obstetrics and Gynecology: General Gynecology. Philadelphia, Mosby-Elsevier, 2007:85.

20. Robert J. Kurman; Blaustein’s Pathology of Female genital tract. 6th edition, New York, springer 2011 Page No (157).

21. Ross M.H, Romrell L.J. Kaye GI, Histology A text and atlas 1995; (edi) 3rd edition, Williams and Wilkins, P 696.

22. Rosai and Ackerman's Surgical Pathology-Tenth Edition (Volume 2),Uterus-cervix, Normal anatomy, pg 1436.

23. IARC (2007) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans,volume 90: Human Papillomaviruses. IARC Press: Lyon.

24. Robert J. Kurman; Blaustein's Pathology of Female genital tract. 6th edition, New York, springer 2011 Page No (162).

25. Rosai and Ackerman's Surgical Pathology-Tenth Edition (Volume 2),Uterus- Cervix, Normal anatomy, pg 1437-1443.

26. Tomita Y , Shirasawa H, Sekine H,Simizu B. Expression of the human papilloma virus type 6bL2 open reading frame in Escherichia coli:L2 β-galactosidase fusion proteins and their antigenic properties. Virology 1987; 158: 8-14.

27. Howley PM (1991)Papilloma virinae and their replication.In: Fields BN,Knipe DM (eds)Fundamental virology.Raven ress,Newyork,pp 743-770.

28. Crum CP: Contemporary theories of cervical carcinogenesis: the virus, the host, and the stem cell. *Mod Pathol* 2000; 13:243-251.

29. Milde-Langosch K, Riethdorf S, Loning T: Association of human papillomavirus infection with carcinoma of the cervix uteri and its precursor lesions: theoretical and practical implications. *Virchows Arch* 2000; 437:227-233.

30. Stoler MH: Human papillomaviruses and cervical neoplasia: a model for carcinogenesis. *Int J Gynecol Pathol* 2000; 19:16-28.

31. Zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2: 342-50.

32. Muñoz N, Castellsague X, de Gonzales AB, and Gissman L (2006) Chapter 1: HPV in the etiology of human cancer. Vaccine 24 Suppl 3: S3/1-10.

33. Stanley M (2010) Pathology and epidemiology of HPV infection in females. Gynecol Oncol 117(2 Suppl): S5-10.

34. Munoz N,Bosch Z,de Sanjose S,Herrero R,Castellsangue X,Shah KV,Snijder fPIF,Meijer CI, Epidemiologic classification of human papilloma virus types associated with cervical cancer.N Engl Journal Med 2003, 348; 518 - 527.

35. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Cage JC, and Castle PE (2011)Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst 103: 368-83.

36. IARC (2012) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans,volume 100B: Biological agents. IARC Press: Lyon

37. De Villiers EM, Fauquet C, Broker TR et al (2004) Classification of papillomaviruses. Virology 324(1):17–27

38. Schiffman M, Wentzensen N,Kinney W, Gage JC,Castle PE. Human papilloma virus testing in the prevention of cervical cancer.Natl cancer inst. 2011 oct 5 volume 103.(19), 1482.

39. Connell PP et al (1999) Race and clinical outcome in endometrial carcinoma. Obstet Gynecol 94:713–720

40. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995; 87(11): 796-802.

41. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992; 79(3): 328-37.

42. Clifford G, Franceschi S, Diaz M et al (2006) Chapter 3: HPV type distribution in women with and without cervical neoplastic diseases. Vaccine 24(Suppl 3):S26–S34

43. Robert J. Kurman; Blaustein's Pathology of Female genital tract. 6th edition, Precancerous lesions of cervix.New York, springer 2011 Page No (201).

44. Smith JS, Lindsay L, Hoots B et al (2007) Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 121(3):621–632.

45. Klaes R,Friedrich T,Spitkovsky D,et al.Overexpression of $p^{16INK4a}$ as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri.International Journal of cancer.2001;92;276-84.

46. Lloveras B, Clavero O, Alejo M, Ordi J, et al: The distribution of HPV types in invasive cervical carcinoma. Analysis of 9760 cases. *Lab Invest* 2009; 89(Suppl 1):225A.

47. Margaret A Stanley. Epithelial cell responses to infection with Human papilloma virus. Clin microbiol Rev. April 2012 ;25(2); 215 – 222.

48. Ham J Dostatni N,Ganthier JM etal.The papilloma virus E2 protein: A factor with many talents.Trends Biochem sci 1991;16:440.

49. Ward P,Coleman DV, Malcolm ADB.Regulatory mechanisms of the papilloma viruses.Trends Grnet;1989;5:97.

50. Scheffner M,Takahashi T, Huibregtse JM et al.Interaction of the human papilloma virus type16E6 on coprotein with wild-type and mutant human p53 proteins.J Virol 1992;66:100.

51. Keating JT, Cviko A, Riethdorf S, Riethdorf L, Quade BJ, Sun D, *et al*. Ki-67, Cyclin E, and p16 INK4a Arc complimentary surrogate biomarkersfor human papilloma virus – related cervical neoplasia. AmericanJournal of Surgical Pathology 2001;25:884-91.

52. Crum, Nucci ,Lee. Diagnostic Gynaecologic and obstetric pathology. Elsevier. Cervical squamous neoplasia.2011.pg 247 and 260.

53. Kahn JA (2009) HPV vaccination for the prevention of cervical intraepithelial neoplasia (CIN),N Engl J Med 361:273

54. Rosai and Ackerman's Surgical Pathology-Tenth Edition (Volume 2),Uterus- Cervix, Normal anatomy, pg 1436-1451.

55. Berrington de Gonzalez A, Sweetland S, and Green J (2004) Comparison of risk factors for squamous cell and adenocarcinomas of the cervix: a meta-analysis. Br J Cancer 90:1787-91.

56. Castellsague X, Diaz M, de Sanjose S, Muñoz N, Herrero R, Franceschi S, et al. (2006) Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications forscreening and prevention. J Natl Cancer Inst 98: 30

57. Smith JS, Green J, Berrington de Gonzalez A et al (2003) Cervicalcancer and use of hormonal contraceptives: a systematic review.Lancet 361(9364):1159–1167

58. Appleby P, Beral V, Berrington de Gonzalez A et al (2007) Cervicalcancer and hormonal contraceptives: collaborative reanalysis of individualdata for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies.Lancet 370(9599):1609–1621

59. Fordyce EJ, Wang Z, Kahn AR et al (2000) Risk of cancer among women with AIDS in New York City. AIDS Public Policy J 15(3–4):95–104

60. Wright TC, Kuhn L (2006) Immunosuppression and the cervix: human immunodeficiency virus (HIV). In: Jordan JA, Singer A (eds) The cervix. Blackwell, Malden, pp 450–517 (2006)

61. Parikh S, Brennan P, and Boffetta P (2003) Meta-analysis of social inequality and the risk of cervical cancer. Int J Cancer 105: 687-91.

62. Kahn JA, Lan D, and Kahn RS (2007) Sociodemographic factors associated with high-risk human papillomavirus infection. Obstet Gynecol 110: 87-95.

63. Chung, S.H.; Wiedmeyer, K.; Shai, A.; Korach, K.S.; Lambert, P.F. Requirement for estrogen receptor alpha in a mouse model for human papillomavirus-associated cervical cancer. *Cancer Res. 2008, 68,* 9928–9934

64. McKenna, D.J.; Patel, D.; McCance, D.J. miR-24 and miR-205 expression is dependent on HPV onco-protein expression in keratinocytes. Virology 2014, 448, 210–216.

65. Yu, Y.; Zhang, Y.; Zhang, S. MicroRNA-92 regulates cervical tumorigenesis and its expression is upregulated by human papillomavirus-16 E6 in cervical cancer cells. *Oncol. Lett.* **2013**, *6*, 468–474

66. Krivak TC, Rose GS, McBroom JW, Carlson JW, Winter WE, 3rd, and Kost ER (2001)Cervical adenocarcinoma in situ: a systematic review of therapeutic options andpredictors of persistent or recurrent disease. Obstet Gynecol Surv 56: 567-75.

67. Buckley CH, Butler EB, Fox H. Cervical intraepithelial neoplasia.J Clin Pathol 1982;35:1-13.

68. Broder S. From the National institutes of Health .Rapid communication-The Bethesda system for reporting cervical / vaginal cytologic Diagnosis – Report of the 1991 Bethesda workshop. JAMA 1992;267:1892.

69. World Health Organisation Classification of tumors.Tumors of the Breast and Female genital organs.IARC press, Lyon , 2003. Pg No 260 – 271.

70. Ylitalo N, Josefsson A, Melbye M, Sorensen P et al. (2000) A prospective study showing long-term infection with human papillomavirus 16 before the development of cervical carcinoma in situ. Cancer Res 60: 6027-32.

71. Mitchell MF, Tortolero-Luna G, Wright T et al (1996) Cervical human papillomavirus infection and intraepithelial neoplasia:a review. J Natl Cancer Inst Monogr 21:17–25

72. Christopher D.M. Fletcher.Diagnostic histopathology of tumors.Fourth edition; volume 1;Elsevier ; page no :818.

73. Sternberg's Diagnostic surgical pathology ;Sixth edition ; volume II ; Wolters kluwer ;page no 2400 – 2402.

74. John A-Rock , Howard W. Tones Telinde's operative Gynecology 10th Edition, Wolters Kluwer, 1208-1225

75. Wright TC Jr,Massad LS, Dunton CJ,Spitzer M,Wilkinson EJ,Solomon D., AmericanSoceity for colposcopy and cervical pathology sponsored consences conference.2006 consences guidelines for the management of women wit abnormal cervical cancer screening tests.Am J Obstet Gynecl.2007;197(4):346-355.

76. Robert W Shaw, David leuster, Ash monga. Gynacology, 4th Edition 2011, Elsevier 587-596.

77. Koliopoulos G, Sotiriadis A , Kyrgiou M, et al.Conservative surgical methods for FIGO stage IA2 squamous cervical carcinoma and their role in preserving women's fertility.Gynecol Oncol 2004;93(2):469-473.

78. Hopkins MP, Morley GW: Radical hysterectomy versus radiation therapy for stage IB squamous cell cancer of the cervix. *Cancer* 1991; 68:272-277.

79. Morley GW: Pelvic exenterative therapy and the treatment of recurrent carcinoma of the cervix. Semin Oncol 1982; 9:331-340.

80. Henriksen E: The lymphatic spread of carcinoma of the cervix and the body of theuterus. Am J Obstet Gynecol 1949; 58:924-942.

81. Tellis CJ, Beechler CR: Pulmonary metastasis of carcinoma of the cervix. A retrospective study. Cancer 1982; 49:1705-1709.

82. Uqmakli A, Bonney Jr WA, Palladino A: The nonlymphatic metastases of carcinoma of the uterine cervix. A prospective analysis based on laparotomy. Cancer 1978; 41:1027-1033.

83. Wentzensen N, von Knebel Doeberitz M. Biomarkers in cervical cancer screening. Dis Markers.2007;23(4):315–330. This descriptive review highlights the biological underpinning and clinical implications of current and future biomarkers in cervical cancer screening.

84. Kalof AN,Evans MF, summons Arnold L, Beatty BG,cooper K. $p^{16INK4a}$ immunoexpression and HPV insitu hybridation signal patterns: potential markers of high grade cervical intraepithelial neoplasia.Am J surg pathol 2005 May ; 29(5) : 674-679.

85. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol Biomarkers Prev. 2008;17(10):2536–2545. This review is a comprehensive compilation of mRNA and p16 based biomarkers in cervical cancer screening.

86. Tsoumpou I, Arbyn M, Kyrgiou M, et al. p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev. 2009; 35(3): 210–220.

87. Omori M, Hashi A, Nakazawa K, Yuminamochi T, Yamane T, Hirara S, et al. Estimation of prognoses for Cervical Intraepithelial Neoplasia 2 by $p^{16INK4a}$ immunoexpression and High-Risk HPV In Situ Hybridization signal types. Am J Clin Pathol. 2007;128:208–17.

88. Halloush RA, Akpolat I, Jim Zhai Q, Schwartz MR, Mody DR. Comparison of ProEx C with $p^{16INK4a}$ and Ki-67 immunohistochemical staining of cell blocks prepared from residual liquid-based cervicovaginal material: a pilot study. Cancer. 2008;114(6):474–480.

89. Baak JP, Kruse AJ, Robboy SJ, Janssen EA, van Diermen B, Skaland I. Dynamic behavioural interpretation of cervical intraepithelial neoplasia with molecular biomarkers. J Clin Pathol.2006;59(10):1017–1028.

90. Murphy N, Ring M, Heffron CC, King B, Killalea AG, Hughes C, *et al*. $p^{16INK4a}$, CDC6 and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. J Clin Pathol 2005;58:525-34.

91. Branca M, Giorgi C, Santini D, et al. Survivin as a marker of cervical intraepithelial neoplasia and high-risk human papillomavirus and a predictor of virus clearance and prognosis in cervical cancer. Am J Clin Pathol. 2005;124(1):113–121.

92. Liang J, Mittal KR, Wei JJ, Yee H, Chiriboga L, Shukla P. Utility of $p^{16INK4a}$, CEA, Ki67, P53 and ER/PR in the differential diagnosis of benign, premalignant, and malignant glandular lesions of the uterine

cervix and their relationship with Silverberg scoring system for endocervical glandular lesions. Int J Gynecol Pathol. 2007;26(1):71–75.

93. Yu L, Wang L, Zhong J, Chen S. Diagnostic value of $p^{16INK4a}$, Ki-67, and human papillomavirus L1 capsid protein immunochemical staining on cell blocks from residual liquid-based gynecologic cytology specimens. Cancer Cytopathol. 2010;118(1):47–55.

94. Schweizer J, Lu PS, Mahoney CW, et al. Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical precancer and cancer. J Clin Microbiol. 2010;48(12):4646–4648.

95. Ruchi Gupta**,** Radhika Srinivasan**,** Raje Nijhawan**,** Vanita Suri**,** Radha UppalProtein $p^{16INK4A}$ expression in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of uterine cervixIndian journal of pathology and microbiology- 53(1), January – march 2010;7 – 11.

96. Asadi-Amoli F, Alain A, Heidari AB, and Jahan I. Detection of Human Papillomavirus Infection in Inverted Follicular Keratosis Lesions of the Eyelid by Immunohistochemistry Method. Acta Medica Iranica. 2009; 47(6): 435-43

97. M. H. Schiffman, H. M. Bauer, R. N. Hoover et al., “Epidemiologic evidence showing that human papillomavirus infectioncauses most cervical intraepithelialneoplasia,” Journal of theNational Cancer Institute, vol. 85, no. 12, pp. 958–964, 1993.

98. N. F. Schlecht, S. Kulaga, J. Robitaille et al., “Persistent human papillomavirus infection as a predictor of cervical intraepithelialneoplasia,” Journal of the American Medical Association, vol. 286, no. 24, pp. 3106–3114, 2001.

99. M. Schiffman, P. E. Castle, J. Jeronimo, A. C. Rodriguez, and S. Wacholder, “Human papillomavirus and cervical cancer,” Lancet, vol. 370, no. 9590, pp. 890– 907, 2007.

100. Riou G, Favre M, Jeannel D, Bourhis J, Le Doussal V, Orth G: Association between poor prognosis in early-stage invasive cervical carcinomas and non- detection of HPV DNA. Lancet 1990; 335:1171-1174.

101. R. Klaes, A. Benner, T. Friedrich et al., “$p^{16INK4a}$ immunohistochemistry improves inter observer agreement in the diagnosis of cervical intraepithelial neoplasia,” American Journal of Surgical Pathology, vol. 26, no. 11, pp. 1389- 1399, 2002.

102. Matsukura T, Sugase M (1995). Identification of genital humanpapillomaviruses in cervical biopsy specimens: segregationof specific virus types in specific clinicopathologic lesions.Int J Cancer, 61, 13-22.

103. Tae Hun Kim, Jee Hye Han, Eun Shin, Jae Hong Noh et al:Clinical Implication of p16, Ki-67, and Proliferating Cell Nuclear Antigen Expression in Cervical Neoplasia: Improvement of Diagnostic Accuracy for High-grade Squamous Intraepithelial Lesion and

Prediction of Resection Margin Involvement on Conization Specimen Journal of Cancer Prevention 2015;20:70-7

104. Maria Benevolo, Marcella Mottolese, Ferdinando Marandino et al Immunohistochemical expression of $p^{16INK4a}$ is predictive of HR-HPV infection in cervical low-grade lesions. Modern Pathology (2006) **19,** 384–341.

105. Purnima Poste, Anuradha Patil d Sainath K. Andola. Incidence of Neoplastic Cervical Pathologies Recorded at a Medical College.IAASR[2][2][2015] 018 035

106. Urmila Banik , Pradip Bhattacharjee ,Shahab Uddin Ahamad ,Zillur Rahman.Pattern of epithelial cell abnormality in Pap smear: A clinicopathological and demographic correlation.CytoJournal 2011, 8:8

107. Kamna Gupta, Nisha Puniya Malik, Veena K Sharma, Nidhi Verma et al: Prevalence of cervical dysplasia in western Uttar Pradesh.J Cytol. 2013 Oct - Dec; 30(4): 257–262.

108. Sophia S. Wang, Marcus Trunk, Mark Schiffman, Rolando Herrero et al: Validation of $p^{16INK4a}$ as a Marker of Oncogenic Human Papillomavirus Infection in Cervical Biopsies from a Population-Based Cohort in Costa Rica.Cancer Epidemiol Biomarkers Prev. 2004 Aug;13(8):1355-60.

109. Geok Chin Tan, Sydee Norlatiffah, N. Akmal Sharifah, Ghazali Razmin et al: Immunohistochemical study of $p^{16INK4A}$ and surviving expressions in cervical squamous neoplasm. Indian Journal of Pathology and Microbiology – 53 (1), January – March 2010.

110. 110.Eun Ji Nam, Jae Wook Kim, Jong Wook Hong, Hyoung Sun Jang et al. Expression of the $p^{16INK4a}$ and Ki-67 in relation to the grade of cervical intraepithelial neoplasia and high-risk human papillomavirus infection.J Gynecol Oncol. 2008 Sep; 19(3): 162–168.

111. Vatsala misra, SC Gupta , Anjali goel, PAsingh. Reclassification by mucin histochemistry of carcinoma cervix uteri. Indian J pathol. Microbial.40(4): 463 – 468;1997.

112. Adisorn Jedpiyawongse, Patcharin Homcha-em, Anant Karalak, Petcharin Srivatanakul. Immunohistochemical Over expression of p16 Protein Associated with Cervical Cancer in Thailand. Asian Pacific Journal of Cancer Prevention, Vol 9, 2008

113. Sano T, Oyama T, Kashiwabara K, et al (1998). Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am JPathol, 153, 1741-8.

114. Galina Volgareva , Larisa Zavalishinina ,Yulia Andreeva ,Georgy Frank et al. Protein as a marker of dysplastic and neoplastic alterations in cervical epithelial cells.BMC Cancer 2004, 4:58.

115. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, Dallenbach- Hellweg G, Schmidt D, von Knebel Doeberitz M: Over expression of $p^{16INK4a}$ as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer2001,92:276-284.

116. Srivastava S. $P^{16INK4a}$ and MIB1: an immunohistochemical expression in preneoplasia and neoplasia of the cervix.Indian patol Microbiol.2010 Jul – Sep;53(3):518 24.

117. Ishikawa M, Fujii T, Saito M, Nindl I, Ono A, Kubushiro K, *et al*. Over expression of p16INK4a as an indicator for human papillomavirus oncogenic activity in cervical squamous neoplasia. Int J Gynecol Cancer 2006;16:347-53.

118. Riou G, Favre M, Jeannel D, Bourhis J, Le Doussal V, orth G: Association between poor prognosis in early-stage invasive cervical carcinomas and non- detection of HPV DNA. *Lancet* 1990; 335:1171-1174.

119. Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S,Ahmadi SA, Sanii S, Hashemi Chelavi L. Potential diagnostic value of P16expression in premalignant and malignant cervical lesions. J Res Med Sci2012; 17(5): 428-33.

ANNEXURE I

PROFORMA

Demographic details:-

1. Name : Date:

2. Age :

3. 0P / IP NO :

4. Parity :

5. Symptoms :

Clinical Diagnosis:-

VIA / VILI :- Positive / Negative / Not done

Histopathological confirmation and grading of H&E stained section

- Cervical Intraepithelial neoplasia - I (CIN-I)
- Cervical Intraepithelial neoplasia -II (CIN-II)
- Cervical Intraepithelial neoplasia - III (CIN-III)
- Early invasive squamous cell carcinoma of cervix.
- Large cell keratinizing Squamous cell carcinoma of cervix.
- Large cell non keratinizing Squamous cell carcinoma of cervix.
- Small cell non keratinizing Squamous cell carcinoma of cervix.

$p16^{INK4a}$ Expression

Negative

Positive

- Grade 1
- Grade 2
- Grade 3

P16 intensity of reaction :-

- Negative
- Weak
- Moderate
- Strong

MASTER CHART

Sl. No	Gynaec No:	Age	Specimen Type	VIA / VILI	HPE diagnosis	Parity	Symptoms	p16 expression	p16 Intensity
1	Gy1591/14	30	Cervix - Biopsy	Positive	CIN I	P1	WD	- Ve	- Ve
2	Gy1596/14	60	Cervix - Biopsy	Positive	CIN II	P2	MET,PCB	3	S
3	Gy1598/14	35	Cervix - Biopsy	Positive	CIN II	NP	WD	2	M
4	Gy1599/14	38	Cervix - Biopsy	Positive	CIN III	P2	WD	3	S
5	Gy1755/14	50	Cervix - Biopsy	ND	CIN III	P3	WD,PCB	3	S
6	Gy1837 / 14	30	Cervix - Biopsy	Positive	CIN I	P2	WD	1	M
7	Gy1870/14	55	Cervix - Biopsy	ND	CIN I	P2	WD	2	M
8	Gy1911/14	70	Cervix - Biopsy	ND	SCC - large cell keratinizing type	P4	PMB	2	M
9	Gy1958/14	48	Cervix - Biopsy	ND	SCC -large cell non keratinising type	P2	MET , PCB	3	S
10	Gy2070	55	Cervix - Biopsy	Positive	SCC - large cell keratinizing type	P2	PMB	3	S
11	Gy2112	38	Cervix - Biopsy	ND	SCC - large cell keratinizing type	P1	PCB, MET	3	W
12	Gy2296	60	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P4	PMB	3	S
13	Gy2315	34	Cervix - Biopsy	Positive	CIN I	P2	WD	- Ve	- Ve
14	Gy2433	48	Cervix - Biopsy	ND	CIN I	P2	MET	- Ve	- Ve
15	Gy2442	35	Cervix - Biopsy	ND	CIN I	P1	WD	- Ve	- Ve
16	Gy2483	45	Cervix - Biopsy	Positive	CIN I	P1	WD	2	M
17	Gy2500	52	Cervix - Biopsy	ND	CIN I	P2	WD	- Ve	- Ve
18	Gy2503	27	Cervix - Biopsy	ND	CIN I	NP	WD	- Ve	- Ve
19	Gy2631	56	Cervix - Biopsy	Positive	SCC - large cell non keratinizing typE	P2	PMB	3	S
20	Gy2984	30	Cervix - Biopsy	Positive	CIN I	P1	MET	3	S

Sl. No	Gynaec No:	Age	Specimen Type	VIA / VILI	HPE diagnosis	Parity	Symptoms	p16 expression	p16 Intensity
21	Gy3045	36	Cervix - Biopsy	Positive	CIN I	P2	WD	- Ve	- Ve
22	Gy3046	45	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P3	PCB , WD	3	S
23	Gy3061	57	Cervix - Biopsy	ND	CIN I	P3	WD	- Ve	- Ve
24	Gy3062	33	Cervix - Biopsy	ND	CIN I	P1	WD	- Ve	- Ve
25	Gy3077	43	Cervix - Biopsy	ND	CIN I	P2	WD	- Ve	- Ve
26	Gy3242	70	Cervix - Biopsy	ND	CIN II	P3	MET	3	S
27	Gy3281	47	Cervix - Biopsy	ND	SCC - large cell keratinizing type	P2	PCB,WD	3	S
28	Gy 3250	45	Cervix - Biopsy	ND	CIN I	P2	WD	- Ve	- Ve
29	Gy3306	42	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P2	PCB, MET	3	S
30	Gy5 / 15	48	Cervix - Biopsy	Positive	CIN I	P1	WD	1	M
31	Gy21/15	35	Cervix - Biopsy	Positive	CIN I	P3	WD	- Ve	- Ve
32	Gy64/15	50	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P3	MET, WD	3	S
33	Gy77/15	60	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P2	PMB	3	S
34	Gy111/15	58	Cervix - Biopsy	ND	CIN III	P3	WD	3	S
35	Gy206/15	65	Cervix - Biopsy	Positive	CIN-I	P3	WD	- Ve	- Ve
36	Gy208/15	65	Cervix - Biopsy	Positive	SCC - large cell non keratinizing type	P3	PMB	3	S
37	Gy219/15	49	Cervix - Biopsy	Positive	CIN II	P3	WD,PCB	- Ve	- Ve
38	Gy336/15	38	Cervix - Biopsy	ND	CIN-I	P1	WD	- Ve	- Ve
39	Gy338/15	60	Cervix - Biopsy	ND	SCC -Early invasive type	P3	WD	3	S
40	Gy343/15	60	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P2	WD,PMB	3	S

Sl. No	Gynaec No:	Age	Specimen Type	VIA / VILI	HPE diagnosis	Parity	Symptoms	p16 expression	p16 Intensity
41	Gy347/15	30	Cervix - Biopsy	Positive	CIN-1	P1	WD	- Ve	- Ve
42	Gy362/15	47	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P2	MET,PCB	3	S
43	Gy530/15	60	Cervix - Biopsy	ND	SCC - large cell non keratinizing type	P3	PMB	3	S
44	Gy632/15	83	Cervix-Biopsy	ND	CIN-III	P3	WD	3	S
45	Gy665/15	65	Cervix-Biopsy	Positive	CIN I	P1	WD	2	M
46	Gy714/15	60	Cervix-Biopsy	ND	SCC -Early invasive type	P2	PMB,WD	3	S
47	Gy722/15	47	Cervix-Biopsy	ND	SCC - small cell non keratinizing type	P2	PCB,US	3	S
48	Gy888/15	60	Cervix-Biopsy	Positive	SCC - large cell non keratinizing type	P4	PMB	3	S
49	Gy928/15	65	Cervix-Biopsy	ND	SCC - small cell non keratinizing type	P2	PMB	3	S
50	Gy982/15	65	Cervix-Biopsy	ND	SCC - large cell non keratinizing type	P3	PMB	3	S
51	Gy1024/15	44	Cervix-Biopsy	ND	CIN-I	P2	WD	1	M
52	Gy1171/15	45	Cervix-Biopsy	Positive	CIN I	P2	WD	- Ve	- Ve
53	Gy1193/15	40	Cervix-Biopsy	Positive	CIN-I	NP	MET	- Ve	- Ve
54	Gy1194/15	50	Cervix-Biopsy	Positive	CIN-I	P2	WD	- Ve	- Ve
55	Gy1226/15	60	Cervix-Biopsy	Positive	SCC - large cell non keratinizing type	P2	PMB	3	S
56	Gy1312/15	35	Cervix-Biopsy	ND	SCC - large cell keratinizing type	P1	WD,PCB	3	S
57	Gy1413/15	73	Cervix-Biopsy	ND	SCC - large cell keratinizing type	P3	PMB	3	S
58	Gy1414/15	45	Cervix-Biopsy	Positive	SCC - large cell non keratinizing type	P2	PCB,WD	3	S
59	Gy1458/15	60	Cervix-Biopsy	ND	CIN II	P2	WD	3	S
60	Gy1508/15	55	Cervix-Biopsy	ND	SCC - large cell non keratinizing type	P3	WD,PMB	3	S

ABBREVIATIONS FOR MASTER CHART

VIA/VILI	:	Visual inspection with acetic acid / Lugol's Iodine
CIN I	:	Cervical intraepithelial neoplasia I
CIN II	:	Cervical intraepithelial neoplasia II
CIN III	:	Cervical intraepithelial neoplasia III
SCC	:	Squamous cell carcinoma
ND	:	Not done(VIA/ VILI)
P1,2,3,4	:	Para 1,2,3,4
NP	:	Nulliparity
WD	:	White discharge
MET	:	Metrorrhagia
PCB	:	Post coital bleeding
PMB	:	Post menopausal bleeding

<u>P16 expression</u>

-ve	:	Negative
1 , 2 , 3	:	Grade 1 ,2 , 3 (p16 expression)

<u>P16 reaction intensity</u>

W	:	Weak
M	:	Moderate
S	:	Strong

ANNEXURE - III

GLOSSARY

LSIL	:	Low grade squamous intraepithelial lesion
HISL	:	High grade squamous intraepithelial lesion
CIN I	:	Cervical intraepithelial neoplasia I
CIN II	:	Cervical intraepithelial neoplasia II
CIN III	:	Cervical intraepithelial neoplasia III
SCC	:	Squamous cell carcinoma
HPV	:	Human papilloma virus
RB	:	Retinoblastoma tumor suppressor gene
PRb	:	Retinoblastoma tumor suppressor gene product
p16	:	p16INK4a
SCJ	:	Squamocolumnar junction
ER	:	Early region
LR	:	Late region
URR	:	Upstream regulatory region
CDK	:	Cyclin dependant kinase
PCR	:	Polymerase chain reaction
ISH	:	Insitu Hybridisation
IHC	:	Immuno histochemistry
MiRNA	:	microRNA
VIA / VILI	:	Visual inspection with acetic acid / Lugol's Iodine